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BUFFER PROCESSES IN THE METABOLISM OF SUCCULENT PLANTS.[1]

BY

JENNY HEMPEL.

INTRODUCTION.

NUMEROUS investigations have in the course of years shown, that the conditions under which the various chemical processes take place, both in living tissue and in vitro, are of a highly restricted character. Temperature and moisture, for instance, exert a determinative effect upon the course of the processes in question, these taking place only within certain limits of the factors mentioned, and even then differing greatly in degree. Many other factors of determinative influence might also be mentioned. The one with which we are here chiefly concerned, however, is the reaction of the substrate in which the processes referred to take place. It has long been known that the presence or absence of acid and base is of decisive importance in the course of many processes, and it has even been supposed that the degree of dependence upon these factors might be expressed by means of the quantity of acid or base respectively found in the substrate on titration. Only the investigations of the last decades, however, have succeeded in making clear the true state of the case, and we now find, that the determining feature is not the quantity of free acid or base, but the amount of dissociated acid (base); more correctly: the quantity of hydrogen ions or hydroxyl ions respectively. In order, therefore, to characterise thoroughly the course of a process, it will also be necessary to state under what concentration of hydrogen ions — and thus also of hydroxyl ions — it takes place.

[1]) Abbreviated from a treatise by the author for the degree of Dr. phil. at the University of Copenhagen, entitled: Bidrag til Kundskaben om Succulenternes Fysiologi. En plantefysiologisk Studie.

Hitherto, this question has been but little investigated, at any rate as regards vegetable tissue, and the statements put forward in extant works on the subject are for the most part restricted to the fact that the cell-sap is in all or most cases of a slightly acid nature[1]), whereas the protoplasm gives a slight alkaline reaction.

To arrive at a thorough knowledge of the conditions under which the processes take place in the cell, we should require to know not only the structure of the cell itself in closest detail, but also the nature of the processes associated with each minute, more or less independent division therein, with all factors of importance, including also the concentration of hydrogen ions, in each. With our methods of investigation in their present stage of developement, however, such detailed knowledge is unattainable, and we must therefore be content, for the time being, with an exposition of the main features. There is, it is true, the possibility that the concentrations of hydrogen ions which take place in the different portions of the cell may be altogether dissimilar; if this were the case, then an analysis of the cell-sap as extracted from the leaves by pressure would in reality give us no idea as to the actual conditions in the tissue. Nevertheless, pending the developement of improved methods for research, such investigations will still be of interest, as with a reasonable degree of regularity in the results thus obtained, there will be considerable probability of our having thereby arrived at a correct view of what takes place in the living tissue.

The purpose of the investigations hereinafter described is to determine the concentration of hydrogen ions in the cell-sap extracted from the leaves, and its relation to the quantity of acid found on titration of

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[1]) Thus, for instance, Gaudichaud (Des sucs séveux acides, et de quelques excrétions alcalines. Comptes rendus **27**. 37 (1848)), who writes: "... qu'il résulte des expériences nombreuses que j'ai faites avec les papiers chimiques sur les tiges, les racines, les feuilles et les parties des fleurs et des fruits que tous les fluides séveux ou noutritifs de la circulation générale des végétaux sont acides ...." J. Sachs also found that the sap of most cells was acid in character; he noted, however, that certain tissues always exhibited alkaline reaction: "nämlich in den dünnwandigen Zellen, welche bei vollständig ausgebildeten Gefässbündeln krautiger Pflanzentheile zwischen den Baste und den Gefässröhren liegen". (Ueber saure, alkalische und neutrale Reaktion der Säfte lebender Pflanzenzellen. Bot. Zeitung. **20**. 264 (1862)).

the same. An endeavour will also be made to explain how such relation arises, and the importance of the same. For the elucidation of these questions, plants subjected to the most widely different external conditions have been employed.

As indicated in the title, the experiments have been especially directed towards investigation of succulent plants, these being, with their well-known high acid content, and the considerable quantitative variation of same in the vegetative parts, *a priori* particularly suited to the present purpose.

On titrating the juice of a succulent, we obtain a value indicative of comparatively high concentration of hydrogen ions; so high indeed, that presuming all or most of the acid to be dissociated — and it must be remembered that we have here to deal with fairly strong acids — such concentration would be detrimental to the protoplasm. It would therefore be reasonable to suppose that some other factors come into play, and that the concentration of hydrogen ions actually taking place is in reality lower than that indicated. Our experiments show that this is in fact the case.

I. PREVIOUS INVESTIGATIONS AS TO THE METABOLISM OF SUCCULENT PLANTS.

The periodical variations in the acid content of succulents was known as far back as the commencement of the previous century. The earliest mention of the phenomenon dates from 1813, when B. Heyne[1]) found that the leaves of Cotyledon (Bryophyllum) calycina were acid as sorrel in the morning, but lost their sour taste as the day wore on, becoming almost bitterish towards evening.

Following up the hint here given, H. F. Link[2]) carried out some years later a series of experiments bearing on the question, and arrived at the same result, not only in the case of Bryophyllum calycium, but also with regard to various other plants, some of which exhibited this peculiarity in a lesser, others in a higher degree.

[1]) The Transactions of the Linnean Society of London. **11**. 213 (1815).

[2]) Jahrbücher der Gewächskunde von Sprengel, Schrader und Link. **1**. 73 (1820).

A considerable period now elapsed before the investigations were again taken up, this time by Adolph Mayer[1]), in 1875, who likewise commenced with the study of Bryophyllum calycium. He showed that a shoot of this plant will, in the dark, reduce the space of air within which it is confined, whereas on exposing the plant to the light, the space is increased, and this as long as the leaves contain acid. The increase of air space during exposure to light is due to a developement of oxygen, which in succulents may even take place when surrounded by a medium from which all carbonic acid has been removed[2]); always provided, however, that acid has been allowed to accumulate by previous confinement in darkness. Mayer further showed, that the acid is worked up into carbohydrates when the plant is exposed for some time to the light of the sun.

Liebig[3]) was of opinion, that this developement of the acids into carbohydrates formed a link in the assimilation of carbonic acid; this theory was, however, opposed by Hugo de Vries[4]), who closely investigated the physiology of succulent plants, and has in several respects elucidated the same from new points of view. He was the first to maintain that both the formation[5]) and the decomposition[6]) of acids are continually going on, and that the accumulation or loss of acid is dependent upon the relation between these two processes. Decomposition is greatly advanced by the effect of light, without regard to the nature of the rays — a fact which he advanced in support of his theory to the effect that acids do not form intermediate products in the assimilation of carbonic acid. Later on, P. Warburg[7]) also found that the nature of the rays was immaterial, whereas Mayer and G. Kraus[8]) maintained that only rays affecting the assimilation

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[1]) Ber. d. d. chem. Ges. **8**. 1088 (1875). Landw. Versuchsstat. **18**. 410—452 (1875). **21**. 278 (1878). **30**. 217 (1884). **51**. 335 (1899).

[2]) This feature was already observed by Saussure among succulents (Opuntia). (Saussure: Recherches chimiques sur la végétation. 76 ff. (1804).

[3]) Justus von Liebig: Die Chemie in ihrer Anwendung auf Agricultur und Physiologie. **49** (1862).

[4]) Bot. Zeitung **42**. 357 (1884).

[5]) Both de Vries and A. Mayer showed that the greater part of the acid in Crassulaceæ is malic acid.

[6]) *l. c.* 339.

[7]) Untersuchungen aus dem Bot. Institut zu Tübingen. **2**. 79 (1886—1888).

[8]) Abhandl. d. Naturforschenden Ges. zu Halle. **16**. 40, 62 (1886).

of carbonic acid were of any importance in this respect. That the decomposition of the acids is a purely oxydizing process was demonstrated by P. Warburg[1]) from the fact that it is furthered or retarded by augmented or diminished supply of oxygen. He further showed, that the intensifying effect of increasing temperature upon the decomposition of the acid both in light and in darkness — already observed by Link, Mayer, De Vries, and others — should be regarded as indicative of intensified vital, and thus also respiratory, processes.

The formation of acids was regarded by de Vries[2]) as a reaction due to stimulation; he considered the daily exposure to light as required to induce the process, albeit this takes place at night. This theory; no acid secretion without previous exposure to light, was, however, subsequently demolished both by Warburg and Kraus.

This last-named writer had first[3]) adopted Liebig's assimilation hypothesis, but showed, in a later work[4]), that the malic acid of the Crassulaceæ should be regarded as a product of oxidation; the mother-substance of the malic acid he supposed to be the carbohydrates formed during the day by assimilation of carbonic acid. In support of this theory he advanced, *inter alia*, the fact that the quantity of acid formed at night increases with increased intensity of assimilation during the previous day[5]). On the other hand, when the plant has previously been exposed to light in an atmosphere devoid of carbonic acid, the quantity of acid formed will be but slight.

As to what became of the malic acid, he expressed the opinion that this was partly deposited in the form of malate of calcium, some portion of it probably also going to form carbohydrates; he has, however, made no attempt to ascertain how this takes place.

The investigations carried out by E. Aubert[6]) were particularly concerned with the production and absorption of oxygen and carbonic acid in succulents. In common with several earlier

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[1]) Abhandl. d. Naturforschenden Ges. zu Halle. **16**. 89 (1886).
[2]) *l. c.* 339.
[3]) *Ibid.* 26 (1884).
[4]) *Ibid.* **16**. 30 (1886).
[5]) *l. c.* 32 (1886).
[6]) Comptes rendus. **112**. 674 (1891).

writers, he considered the mode of respiration among succulent plants as an economic advantage, the interchange of air in thick, fleshy leaves being necessarily a more difficult matter than in thin leaves with a surface area large in proportion to their volume. In agreement with this, he showed than an inverse relation existed between the intensity of respiration and the degree of fleshiness of the leaves of the various succulent species[1]), with a direct relation between the latter factor and the amount of acid which can be accumulated in the leaves[2]). The process of acid decomposition could, he considered, take place in two ways, determined either by the influence of temperature or by that of light. A rise of temperature should occasion a developement of carbonic acid at the expense of the accumulated malic acid[3]), and the influence of light give rise to a splitting up of the acid, accompanied by developement of oxygen[4]). This view was, however challenged by C. Gerber[5]), who maintained that the decomposition of the acid, whether in light or darkness, was associated with developement of carbonic acid, and that this carbonic acid was, during exposure to light, directly utilised for the process of assimilation. He also demonstrated, as Aubert had done, the dependence of the respiratory quotient upon temperature.

In 1893, the work of the mentioned writers was supplemented by the results of Purjevics'[6]) investigations, which proved, *inter alia*, by nutrition experiments with glucose, cane sugar, glycerine, etc. that the intensity of the formation of acid is dependent upon the carbohydrates present. On feeding his plants with acid instead of carbohydrates, he was able to observe an increased respiratory quotient. He then laid down, as the main result of his researches, that the acid is, as most of the earlier writers had opined, to be regarded as an oxidation product of the carbohydrates, and that it may be further split up for instance by the influence of light, and of increased temperature, when it gives off material for the assimilation of carbonic acid.

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[1]) Revue Générale de Botanique. **4**. 374 (1892).
[2]) *l. c.* 384.
[3]) *l. c.* 302.
[4]) *l. c.* 500.
[5]) Annales des Sciences Naturelles. VIII Série. **4**. 151 (1896).
[6]) Quoted from the summary given in Bot. Centralb. **58**. 368 (1894); the original work is in Russian.

As to how the actual decomposition of the acid takes place, this the writer in question had not observed; he is of opinion, however, that the secretion of carbonic acid is effected by for mation of other volatile acids, as he was able to discern that the quantity of volatile acids increases during the process of decomposition. Among these, he continually found acetic acid, and possibly formic acid.

That the decomposition of acid actually does take place by formation of volatile acids was again shown by H. A. Spoehr[1]) who found that a diluted malic acid solution in vitro could be decomposed in sunlight. The process is greatly intensified by adding a small quantity of those substances in the cell-sap (*in casu* cactus) which are precipitated by alcohol. Whether these substances be employed in the raw, boiled or calcined state, their catalytic effect remains the same, whereas the alcoholic precipitate when cleansed by dialysis has no accelerating effect upon the process. From this Spoehr concluded, that it must be the influence of the salts in the plant juice which here makes itself apparent, and that there can be no question of any enzymatic effect. In the malic acid solution exposed to light, Spoehr was able to discern oxalic acid, glycolic acid, formic acid, and acetic acid, besides carbonic acid; in addition, formaldehyde and acetaldehyde were also found. Both for the malic acid and tartaric acid he believed the course of the process to commence with the separation of two CO_2 groups, the alcohol thus formed being further oxydised to acetaldehyde and thence to acetic acid. This would then again be oxydised by way of methylalcohol and formaldehyde to formic acid, which again should be transformed to carbonic acid and water. The oxalic acid would be formed by oxidation of acetaldehyde. The glycolic acid should be decomposed into carbonic acid and methylalcohol, which again should be further oxydised. The fact that the decomposition of the acid proceeds rapidly at first, and is subsequently retarded, is explained by this writer as due to the greater constancy of acetic and formic acid.

By exposing the acid for a sufficient length of time to the light, he found that the quantity of carbonic acid formed was so great as very nearly to equal the calculated amount, all the remaining volatile substances having become decomposed.

[1]) Biochem. Zeits. **27**. 95 (1913).

As a summary of the foregoing results we may assert that succulents form (and accumulate) organic acid at the expense of carbohydrates, and that the acid when decomposed produces carbonic acid, which may be utilised for the assimilation of carbonic acid. The acids must thus be regarded as intermediate products in the respiratory process, and any accumulation of the former will be determined by various factors such as light, temperature, state of nutrition, etc.

II. THE PRESENT INVESTIGATIONS.

A. METHODS AND MATERIAL.

The material investigated consisted partly of various succulents, partly seedlings of Lupinus albus, with some lemons, and the pitcher-fluid of certain Nepenthes species.

The succulents examined were almost all obtained from the Botanical Garden. The following species were used:

Crassulaceæ:

Diotostemon Hookeri Salm-Dyck.
Cotyledon linguæfolia Lem.
Cotyledon coruscans Haw.
Cotyledon obvallata Haw.
Crassula obovata Haw.
Crassula lactea Soland.
Rochea falcata D. C.

Compositæ:

Kleinia (cuneifolia D. C.?).

Aizoaceæ:

Mesembrianthemum echinatum Lam.
Mesembrianthemum linguiforme Haw.
Mesembrianthemum Lehmannii Eckl. & Zeyk.

Liliaceæ:

Aloe arborescens Mill.
Aloe cymbæfolia Schrad.
Aeonium Haworthii Webb. & Berth.

The plants were kept in a large glasshouse — without heating apparatus — in the experimental nursery of the Carlsberg Laboratory. For darkening the plants, a cylinder of black pasteboard was used, which effectually excluded all light.

When exposing a plant to the light, it was placed immediately behind the glass wall of the house on the south side, in such a manner that no shadow could fall on it. The plants were watered 48 or at latest 24 hours before being subjected to experiment. The temperature was noted on a thermograph, but these curves are only included here where the conditions of temperature present features of special interest. No attempt was made however, to measure the intensity of light to which the plants were exposed.

From the variously exposed plants, leaves were taken, weighed, and their cell-sap extracted by pounding in a porcelain mortar with a pestle of the same material. The sap, which in nearly all cases was green owing to the presence of chlorophyll, was centrifuged in a hand apparatus and then filtered. (In the case of some plants, the sap could not be filtered, owing to its more or less slimy composition; I had then to be content with decanting it from the dregs.) In most cases, the sap was after filtration found to be entirely free from chlorophyll, quite clear, or nearly so, and of a pale yellow colour. The few instances in which this was not the case are duly noted in the respective sections dealing with experiments on the different plants (cf. Sections B 1—14).

The sap thus treated was utilised partly for determination of the concentration of hydrogen ions, partly for titration of the amount of acid present, further for determinations of nitrogen, and in some instances for determination of ash. The pounded leaves, together with the solid matter centrifuged off from the cell-sap, were dried to as nearly as possible constant weight at c. 55°. From the value thus obtained, and the original weight, the content of dry matter in the leaves was then calculated, this being stated throughout as a percentage. Strictly speaking, it is of course not absolutely correct to designate the value thus obtained as the "content of dry matter", the matter held in solution in the cell sap itself being here disregarded; the discrepancy is, however, of insignificant magnitude, and will in any case be more or less uniform for all the experiments.

The object of making such determinations of dry matter was to make sure that the plants had not during the experimental period suffered from drought, but had throughout been in a turgescent state. The figures for dry matter and amount of water will be found in Tables 1—14, Section B. These

figures show that the experimental plants were in normal condition at the time; the slight differences apparent in the quantity of water may be taken as expressive of the variability always found in living vegetable matter.

Electrometric determination of concentration of hydrogen ions.

The electrometric determination has been carried out, partly by means of the hydrogen current method laid down at Carlsberg Laboratory[1]), partly according to the method as modified by Hasselbalch[2]).

The degree of accuracy obtainable with the first-named experimental method answers to c. 0.05 in p_H.

Determining concentration of hydrogen ions with Lakmoid paper.

The concentration of hydrogen ions in hydrous solutions can of course be determined, not only by electrometric, but also by colorimetric methods; when dealing with cell-sap, however, it will in many cases be found impossible to employ the colorimetric method as drawn up by S. P. L. Sörensen, owing to the colour present in the cell-saps themselves By means of indicator paper, on the other hand, it is possible to form a fairly accurate idea as to the concentration of hydrogen ions in a cell-sap. My original object in using indicator paper for this purpose was to ascertain whether the crushing of the leaves etc., centrifuging,

[1]) S. P. L. Sörensen: Comptes rend. d. Lab. d. Carlsberg. **8**. 20. (1910).

[2]) Comptes rend. d. Lab d. Carlsberg. **10**. 69. (1911). As an indication of the agreement between the two methods, the following example may be quoted, this showing a particularly marked diserepancy in the measurements. Thus the figures for Cotyledon obvallata were as follows (figures here indicating the resistances used for the separate measurements).

Normal element: (N-E.) 502.2

Hydrogen element (H.E.)
1. 304.4
2. 305.7
3. 304.7
4. 305.0, whence $\pi = 0.6188$ $p_H = 4.88$.

After a sufficiently long exposure to the hydrogen current the following values were found:

N-E. 502.2
H-E. 306.0 $\pi = 0.6208$ $p_H = 4.91$.

filtration and electrometric measurement of the juice, occasioned any alteration in the concentration of hydrogen ions, either by loss of carbonic acid or in other wise. At the suggestion of Prof. Sörensen, therefore, I examined various sorts of indicator paper as produced at the laboratory here, and found Lakmoid paper suitable for the purpose of the experiments here in question. The measurement by means of indicator paper is based on the fact that liquids with different concentration of hydrogen ions will, when applied in drops to the paper, produce different colours; in the case of this particular paper a scale from red per violet to blue. We can then estimate the concentration of hydrogen ions in a liquid by comparing the colour produced by a drop of the same upon the paper with that derived from other liquids whose concentration of hydrogen ions is known.

By comparing this result with that obtained on renewed measurement of the cell-sap by the same method after the various operations of crushing, etc have been carried out, it is possible to ascertain whether any alteration in the concentration of hydrogen ions has taken place or not. It is of course not sufficient to take a single sample; several leaves should be tested in the same manner, as there may always be some difference between the sap of different leaves. The reason above given is, moreover, not the only one for adopting this simple method (for degree of accuracy see Section D) it may also frequently be an advantage to have a means whereby we can rapidly, and without waste of material, ascertain how far a certain plant is in the condition required for purposes of investigation.

In Tables 1—14, Section B, will be found the values thus arrived at for concentration of hydrogen ions. The figures show that the values were as a rule the same before and after pounding the leaves; only in a few instances were some slight differences apparent, rarely amounting to more than 0.1, only in one case to 0.2 and in one to 0.3 in p_H. These slight differences may doubtless be taken as due to the inaccuracy of the method (see Section D); it is of course possible, however, that they may express slight alterations in the composition of the sap during the process of pounding and cleansing.

Lakmoid paper may be used for a range of hydrogen ion concentrations extending from c. $10^{\div 3.8}$ to c. $10^{\div 6}$, or, expressed in p_H, from c. 3.8 to c. 6.

The ordinary lakmoid of commerce may be cleansed according to the method given by Glaser[1]). The paper is prepared by drawing strips of filter paper through the lakmoid solution and hanging them on strings to dry. The process will generally need to be repeated in order to give the paper an even tone.

Titration of the cell-sap.

A weighed portion (between 10 and 30 g.) of the centrifuged and filtered juice was used for titration, this being titrated to different stages. For titration, $^n/_5$ sodium hydroxide and $^n/_5$ hydrochloric acid were used. The titration was first carried to the transition point of the litmus paper — hereinafter referred to as the litmus point — answering to a concentration of hydrogen ions of $10^{\div 6.81}$. In order to attain this point exactly, a mixture — as recommended by S. P. L. Sörensen — consisting of equal parts $^1/_{15}$ mol. prim. potassium phosphate and $^1/_{15}$ mol. sec. sodium phosphate was used, the tint produced by this mixture upon the litmus paper answering to $p_H = 6.81$, which should be that produced by the liquid in question when titrated up to the litmus point. Phenolphtaleïn was then added to the liquid, and this titrated to a certain shade of red corresponding to a hydrogen ion concentration of $10^{\div 9.2}$, hereinafter termed the phenolphtaleïn point. For testing the titration, a borate solution was used, consisting of 0.2 g. mol. boracic acid in 100 cc. norm. sodium hydroxide, diluted with water to 1 litre, to which was added one drop of phenolphtaleïn for each 2 cc. borate solution. Owing to the composition of the juice, the titration brought out, instead of the true phenolphtaleïn red, a series of peculiar muddy-reddish shades (from bluish red to reddish brown) rendering it necessary to add various colour substances to the test solutions (para nitrophenol, methyl violet, Bismarck brown) with some powdered kaolin to cloud the liquid, as the plant juices, on titration to such low concentrations of hydrogen ions as here in question, invariably became highly clouded by principitating matter. The shades of colour shown by the juices on titration to this particular stage varied greatly for the different species of plants, but were generally uniform for one and the same species. Even here,

[1]) F. Glaser: Indikatoren der Acidimetrie und Alkalimetrie (1901).

however, I have occasionally noticed instances of quite considerable difference, for instance when dealing with portions of young leaves as against old (e. g. in Diotostemon).

Furthermore, the quantity of matter precipitated during titration differed greatly for the different species of plants; thus in the case of Rochea falcata and Aeonium Haworthii a very abundant precipitate was formed, whereas in Cotyledon obvallata, Cotyledon coruscans and Mesembrianthemum echimatum, the quantity of precipitate was comparatively slight. Even within one and the same species, however, especially in the case of Rochea falcata, the quantity of precipitate exhibited considerable variation, and would appear to be in some degree dependent upon the quantity of those substances which determine the difference between the figures for titration to the litmus point and to the phenolphtaleïn point. This will be more fully referred to later on.

Endeavours were also made to titrate to a very pale rose tint ($p_H = 8.3$); these however, proved unsuccessful, the colour of the solution itself here predominating in too high a degree.

With experiments upon one and the same species of plant extending over several days I have as a rule employed the same test solution throughout, keeping the latter in darkness during the intervals, and carefully noting, by means of the colour scale given in the "Code des couleurs"[1]) that it had not lost colour in the meantime.

The results of the acid titrations will be found in Tables 1—14, Section B, together with the results of the remaining analyses for juices of the various plants. There are also arranged in a co-ordinate system in Diagrams 1—10, where they appear as ordinates, with the corresponding values for p_H of the juices as abscissæ. The points for the figures obtained by litmus titration (noted as O) lie in more or less smooth curves, whereas the arrangement of those for the values obtained in phenolphtalein titration (noted as □) is altogether irregular.

Determination of Nitrogen in the cell-sap.

The determinations of nitrogen were carried out according to Kjeldahl's method, by decomposing a weighed portion of

[1] P. Klincksieck & Th. Valette: Code des Couleurs (1908).

juice (frequently that used for the acid titration) with conc. sulphuric acid and a small quantity of copper oxide, afterwards treating with permanganate of potassium. The ammonia was distilled over in c. $^n/_7$ sulphuric acid and determined by the usual iodometric acid titration.

Tables 1—14, Section B, show the values found for amount of nitrogen in the juices. From these it will be seen that the quantity of nitrogenous compounds in the sap is not constant for each species of plant, and that the amount does not stand in any definite relation either to the concentration of hydrogen ions or the quantity of acids, or quantity of the substances which determine the difference between the titration figures for litmus and phenolphtaleïn points.

In some few of the first experiments also, determinations were made of the free amino groups present in the nitrogenous substances of the cell-sap, according to S. P. L. Sörensen's[1] formol titration method; it was soon found, however, that it would be fruitless to hope for any certain result in this respect, the nitrogenous substances being present only in very slight quantity in the plant juices.

Analysis of Ash.

A weighed portion of cell-sap (between 50 and 150 g.) was reduced by evaporation, finally in a crucible, to a dry state, the remains being then slowly heated to a glow over a Bunsen burner, until the ash was burnt almost white. A small quantity of hydrochloric acid and water was then added, and the contents of the crucible filtered, the filter paper used being one leaving little ash. The filter with its contents was then washed in boiling water, dried, and heated to a glow. The residue was then mixed with a little hydrochloric acid and water, and added to the dissolved main portion, which was then subjected to usual methods[2] for quantitive determination of silicic acid (weighed as SiO_2) Iron + Aluminium (weighed as Fe_2O_3 + Al_2O_3), Calcium, Magnium (precipitated as oxalates, weighed as CaO and $Mg_2P_2O_7$ respectively), and potassium + sodium (weighed as sulphates)[3].

[1]) Comptes-rendus des travaux du Laboratoire de Carlsberg. **7**. 1 (1907).

[2]) Julius Petersen: Metoder til kvantitativ Analyse. 1903.

[3]) In the case of Cotyledon obvallata, determination of alkalis was in some instances (Exp. 1—6, Table 4 a) omitted. In these experiments, the residue

Tables 2 b, 4 b and 7 b show the values thus found, expressed as absolute quantities calculated per 100 g. juice, and also as milligramme equivalents.

The heated mixture of aluminium oxide and ferrioxide was in all cases almost entirely white, with only a slight brownish-red tinge, showing that iron is only present in small quantities. In transposing the absolute quantity of ash to mg. equivalents, the figure for aluminium oxide, viz; 17.03, has also here been employed. The equivalent figure for ferrioxide is 26.63, but as the iron is only present in small quantities, the error occasioned by taking the equivalent value for aluminium oxide will be insignificant.

In transposing the figures for alkali sulphates, the value for potassium sulphate, 87.18, has been employed, this being somewhat higher that that for sodium sulphate (71.08). A qualitative analysis of the ash from Rochea falcata, Cotyledon obvallata and Cotyledon linguefolia showed a very distinct potassium reaction (potassium precipitated as potassium coboltinitrite) whereas the sodium reaction (sodium precipitated as acid pyro-antimonate) was very indistinct. Reaction with a known quantity of sodium salt showed, that the quantity of sodium present in the juices would at the utmost correspond to but c. 2 mg. sodium per 100 g. of juice. In one of the experiments with Rochea, however, a separation of the alkalis was made, previous qualitative analysis having in this case revealed a stronger sodium reaction. In this instance, the alkalis were first determined together as sulphates. and the potassium then as potassium platinochloride, after the sulphuric acid had been removed in the usual way.

Qualitative determination of Acids precipitating Calcium.

A qualitative analysis of the juice of Rochea falcata was made in the following manner: 80 cc. plant juice, containing 1 g. malic acid (calculated by means of the total quantity of

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from the evaporation of the plant juice was finally heated under spirit blower to constant weight. It was found, however, that the carbonate of potassium formed during combustion partly disappeared under the intense heating, and the method above mentioned was therefore employed instead. The weight arrived at after heating with spirit blower is stated in the last column under the heading of ash.

base in the juice) was used for the analysis. As a test of the method, three similar portions were likewise examined, with the addition to these of 1, 2 and 5 % respectively of the remaining acids precipitating calcium (oxalic acid, tartaric acid and citric acid) calculated in proportion to the quantity of malic acid. The addition of these rendered the juice somewhat opaque, owing to the formation of the calcium salts of the added acids; these were, however, dissolved by adding nitric acid in drops, and slightly heating. Sodium carbonate was then added, until the liquid was just verging on the precipitation point, when the precipitate was made with acetate of lead. After standing for some time, the precipitate was filtered off, washed and decomposed with sulphuretted hydrogen. After filtering off the lead sulphide and boiling away the sulphuretted hydrogen, the liquid was subjected to strong evaporation, acetised, and solid acetate of potassium added to precipitate the tartar. The process of precipitation was accelerated by cooling in ice, and scraping regularly with a spatula; on the following day, the tartar deposit was filtered off and rinsed with water. The tartar was then dissolved in ammonia, and the calcium salt of the tartaric acid precipitated by the addition of calcium acetate. This salt was brought to the crystalline state by treating with acetic acid; after washing, the salt was utilised for the silver-mirror test. In cases where only a very slight deposit of tartar was obtained, this was used directly, after washing, for the test.

The filtrate from the tartar was heated, and precipitated with calcium acetate, always in acetised liquid, whereby a deposit of calcium oxalate was obtained. This salt was filtered off, washed and dissolved in hydrochloric acid, and subsequently identified by the sodium acetate test and — after liberating the oxalic acid — by the copper sulphate test.

The filtrate from the calcium oxalate was ammoniatised to precipitate the phosphoric acid. The precipitate was dissolved in a small quantity of nitric acid and tested with ammonium molybdate. The filtrate from the calcium phosphate was precipitated with two parts of alcohol, whereby a rich white precipitate was obtained, which might consist of the calcium salts from the citric and malic acids. As a test for the citric acid, a portion of this deposit was subjected to Stahre's pentabromaceton reaction, the acid having first been liberated by heating with sulphuric

acid. By this method, it was possible to discern, in those portions of the juice to which the 1, 2 and 5 % of each of the calcium-precipitating acids (oxalic, tartaric and citric) had been added, the presence of the substances in question. In that portion, however, to which only 1 % had been added of each, the oxalic acid was very difficult to discern, whereas the reactions in all the remaining cases were distinct. In that portion of the Rochea sap to which none of these substances had been added, neither oxalic nor tartaric acid could be discovered, while as regards the citric acid, the reaction with Stahre's method was here so indistinct that it must be counted as negative; in the portion to which 1 % citric acid had been added the reaction was very distinct.

The malic acid preparation used for making up the malic acid solutions, was investigated in a similar way.

B. DESCRIPTION OF THE EXPERIMENTS WITH SUCCULENTS.

1. DIOTOSTEMON HOOKERI Salm-Dyck.

All the experiments with this plant were made during the autumn of 1913. On titration, the precipitate was always found to be particularly abundant in the cell-sap of old leaves, in addition to which, the red colour produced upon titration with phenolphtaleïn as indicator was of different shade in the cell-saps of old as compared with younger leaves. This feature was very distinctly apparent in Experiments 6 and 7 (cf. Table 1 a); in the juice obtained from young leaves, there was always a yellowish-brown tinge in the red, whereas the red from the juices of older leaves exhibited a fairly marked bluish tone. The titration was throughout — in this as in all the other plants investigated — far sharper in the case of juice from young leaves, which naturally agrees with the slighter degree of precipitation.

For the six first experiments, one whole plant was used for each portion; in experiment No. 7, four plants were taken, and of these, all the small young leaves were used for a, all the older and larger ones for b.

Experiments 7 a and b show that the concentration of hydrogen ions may differ for the different parts of the plant (in

DIOTOSTEMON HOOKERII SALM-DYCK.

TABLE 1a.

	g. leaves	% dry matter	% water	pH Lakmoïd-test		pH Electrom. measurement		Direct titration values		Litmus cc. n/5 per 100 g. sap	Phenol-phtaleïn cc. n/5 per 100 g. sap	mg. N per 100 g. sap
				before	after	Hassel-balch's method	H-current method	Litmus cc. n/5	Phe-nolpht. cc. n/5			
1. Left in light. Examined 4 Oct. 11 o'c.	31.74	3.33	96.67	4.4	4.4	4.25	4.26	3.93	6.25	20.54	32.67	6.72
2. Left in light. Examined 10 Oct. 3.30. 18 leaves	54.04	4.23	95.77	4.9	4.9	4.86	4.85	1.17	4.11	7.80	27.40	4.45
3. 18 hours in darkness; exam. 11 Oct. 11.30. 14 leaves	45.95	3.63	96.37	4.4	4.4	4.35	4.37	4.34	8.29	17.71	33.84	8.46
4. Left in strong sunlight; exam. 11 Oct. 2.30. 36 leaves	26.02	3.50	96.50	5.4	5.4	—	5.29	0.38	1.37	2.46	8.89	12.41
5. Left in sunlight; examined 13 Oct. 11.20. 21 leaves	40.44	4.22	95.78	5.3	5.3	5.19	5.24	0.71	4.59	3.55	22.95	3.99
6a. 18 hours in darkness; young plant with 39 leaves	18.94	3.35	96.65	4.4	4.4	4.35	4.38	1.82	2.40	17.98	23.72	8.37
6b. Examined 14 Oct. 1 o'c. older plant with 15 leaves	28.69	3.75	96.25	4.4	4.4	4.33	4.39	2.98	7.50	16.80	42.62	8.82
7a. Left in sunlight; 44 small leaves	22.25	4.11	95.89	5.4	5.4	5.55	5.55	0.20	1.51	1.61	12.18	4.90
7b. Examined 15 Oct. 3 30 11 large leaves	23.99	4.50	95.50	5.2	5.2	5.13	5.16	0.62	3.09	4.92	24.52	3.95

the young leaves $p_H = 5.55$, in the older ones $p_H = 5.16$). And in correspondence with this difference in the concentration of hydrogen ions, a considerable difference was also found in the figures for titration, to wit: 1.61 and 4.92 for the litmus values, and 12.18 and 24.52 for phenolphtaleïn.

In a and b on the other hand, the values for p_H and litmus figures were practically in agreement, which of course does not

Fig. 1.

contradict what was noted in the experiment 7 just mentioned, as two different plants were used for 6 a and 6 b.

Table 1 b shows the differences between the litmus and phenolphtaleïn figures in their relation to the age of the leaves,

Table 1 b.

Experiment No.	3	2	7 b	6 b	5	4	7 a	6 a
Weight of leaves	3.3	3.0	2.2	1.9	1.9	0.7	0.5	0.5
Phenolph. ÷ Litmus ..	16.13	19.60	19.60	25.82	19.40	6.43	10.57	5.74
mg. N per 100 g. sap..	8.46	4.45	3.95	8.82	3.99	12.41	4.90	8.37

as estimated from the average weight, which was, in all the experiments save No. 1, calculated from the number af leaves.

There is no absolute decrease in the values for phenolphtaleïn ÷ litmus with decreasing weight of leaves; such was, moreover, hardly to be expected, as the age of the leaves is only to a certain extent determinable from their size. The material can, however, be divided naturally into two groups, as indicated by the double line here separating the younger from the older leaves. A marked dissimilarity is here apparent in the difference mentioned, showing that the leaves, with increasing age, accumulate substances which appear as acids with hydrogen ion concentrations of less than $10^{\div 7}$. The quantity of nitrogenous compounds likewise bears no relation to age or size of the leaves.

2. COTYLEDON LINGUÆFOLIA Lem.

The general arrangement of the experiment will be seen from the table: the following points should, however, be noted: For No. 5, two plants were used, of which each contributed the same quantity to either portion, the leaves being so selected that those used for a and c; b and d respectively, were as far as possible alike in respect of age and position on the stem.

For No. 6, two plants were used; the leaves for a were taken from one side of the stem of either plant, the remainder being utilised for b. During the dark period, the two plants were kept in an ice chest at a constant temperature of 3.5^{0}, after which the leaves for a were picked off and analysed, and the plants with the remaining leaves placed in a glasshouse in a strong light, partly in the sun, at a temperature varying from 20 to 27^{0}.

For Nos. 7—11 a single old plant was used, the leaves being so selected as to maintain the greatest possible degree of uniformity between the different portions.

The figures for acid exhibit considerable variation, suggesting thus a high degree of acid metabolism in the plant, albeit the quantities of acid found are far from equalling those for Kleinia and Rochea *(vide infra)*. The differences between phenolphtaleïn and litmus figures are also very dissimilar, and there is no sort of regularity discernible in their variation as relative to the age of the leaves, which last will be seen from Exp. 5. It is worthy of note that the difference here was slight in a and d, considerably greater in c, and especially in b. Correspondingly, it was

TABLE 2a.

	g. leaves	% dry matter	% water	pH Lakmoïd-test: before	pH Lakmoïd-test: after	pH Electrom. measurement: Hassel balch's method	pH Electrom. measurement: H-current method	Direct titration values: Litmus cc. n/5	Direct titration values: Phenolpht. cc. n/5	Litmus cc. n/5 per 100 g. sap	Phenolpht. cc. n/5 per 100 g. sap	Phenolpht. ÷ Litmus	mg. N per 100 g. sap
1a. Left in light; examined 17 May 1912 1.30	43.86	1.74	98.26	4.6	4.7	4.53	—	2.95	5.26	9.82	17.52	7.70	—
1b. 25 hours in darkness; exam. 18 May 2.30	40.79	1.59	98.41	4.4	4.4	4.23	—	4.03	5.75	15.00	20.73	5.73	2.74
2a. Left in light; examined 29 May 1912 2 o'c.	51.00	2.25	97.75	—	—	4.67	—	2.69	6.33	7.58	17.83	10.25	5.54
2b. Thereafter 24 hours in darkness; exam. 30 May 2 o'c.	47.74	2.10	97.90	—	—	4.00	—	8.30	11.31	24.25	33.04	8.79	—
3. Left in strong sunlight; examined 7 June 1912 3 o'c.	44.07	3.76	96.24	5.5	5.5	5.52	—	0.53	4.08	2.03	15.62	13.59	4.44
4a. Left in light; examined 28 Sept. 1912 3.45	43.70	4.24	95.76	4.8	4.8	(4.73)	—	3.07	9.33	10.43	31.70	21.27	5.33
4b. 48 hours in darkness; examined 30 Sept. 1912	52.82	3.02	96.98	4.3	—	(4.15)	—	8.05	12.54	20.93	32.61	11.68	4.84
5a Left in diffused daylight; Small leaves from top	31.03	1.53	98.47	4.4	4.4	4.20	4.23	3.97	4.82	22.39	27.19	4.80	6.67
5b. exam. 9 Sept. 1913 10.45 Older leaves fr. basal parts	50.38	1.54	98.46	4.7	4.7	4.42	4.43	3.42	6.77	13.68	27.08	13.40	2.94
5c. Left further $4\frac{1}{4}$ h. in strong light, part sun, Small leaves from top	40.19	2.11	97.89	4.7	4.7	4.47	4.48	2.62	4.49	13.10	22.45	9.35	5.21
5d. exam. 3 o'c. Older leaves fr. basal parts	65.64	1.61	98.39	4.8	4.8	4.63	4.64	2.35	3.70	9.40	14.80	5.40	4.81
6a. $23\frac{1}{2}$ hrs. in darkness at 3.5°; exam. 11 Sept. 1913 10 30	82.40	1.88	98.12	4.4	4.4	4.22	4.23	4.68	5.80	18.72	23.20	4.48	7.15
6b. Further $4\frac{1}{4}$ hours in light; examined 11 Sept 2.45	81.08	2.01	97.99	4.8	4.8	4.52	4.53	2.65	3.58	10.60	14.32	3.72	7.31
7. Left in sun; examined 14 August 1914 2 o'c.	84.47	1.51	98.49	—	4.8	4.94	4.92	0.80	1.54	5.33	10.27	4.94	10.00
8. Left in sun; examined 1 Sept. 1914 3.20	111.59	1.70	98.30	4.7	—	4.96	4.99	0.70	1.34	4.67	8.93	4.26	10.28
9. Left $8\frac{1}{2}$ hours in darkness; exam. 8 Sept. 1914 5.30	51.29	1.67	98.33	4.2	—	4.12	4.20	2.01	2.67	19.98	26.54	6.56	12.23
10. Left 3 hours in darkness; exam. 10 Sept. 1914 3.15	59.41	1.67	98.33	4.4	—	4.22	4.29	1.79	2.32	17.72	22.97	5.25	11.98
11. Left $\frac{3}{4}$ hour in darkness; exam. 15 Sept. 1914 3.45	58.71	1.82	98.18	4.5	4.5	4.40	4.43	1.30	1.80	12.90	17.86	4.96	13.09

COTYLEDON LINGUÆFOLIA Lem.

Table 2b.

	g. leaves	% dry matter	% water	Electrom. measurement: Hasselbalch's method	Electrom. measurement: H-current method	Direct titration values: Litmus cc. n/5	Direct titration values: Phenolpht. cc. n/5	per 100 g. sap: Litmus cc. n/5	Phenolpht. cc. n/5	Phenolpht. + Litmus	mg. N	$Al_2O_3 + Fe_2O_3$: g.	$Al_2O_3 + Fe_2O_3$: mg. equivalent	CaO: g.	CaO: mg. equivalent	$Mg_2P_2O_7$: g.	$Mg_2P_2O_7$: mg. equivalent	$K_2SO_4 + Na_2SO_4$: g.	$K_2SO_4 + Na_2SO_4$: mg. equivalent
1. Left in sunlight; exam 18 Nov. 1914	76.41	1.97	98.03	5.35	5.39	0.30	1.15	2.97	11.37	8.40	15.82	0.0516	3.03	0.3367	12.00	0.0348	0.63	0.1491	1.71
2. Left 24 hours in darkness; exam. 19 Nov. 1914	83.46	1.81	98.19	—	5.04	0.50	1.22	5.13	12.04	6.91	12.14	0.0389	2.29	0.3253	11.60	0.0422	0.76	0.1330	1.53
3. Left in light; examined in November 1914	—	—	—	—	—	—	—	—	—	—	—	0.0359	2.11	0.3859	13.76	0.0269	0.48	0.0802	0.92
Mean values...	—	—	—	—	—	—	—	—	—	—	—	—	2.48	—	12.45	—	0.62	—	1.39

found that on titration to phenolphtalein point, an abundant precipitate was formed in b, whereas in the remaining three portions this was but small. From 5 a and b it will be seen that a not altogether inconsiderable difference in the concentration of hydrogen ions may occur between the uppermost young leaves and the older ones at the base of the stem; four hours later, this difference had been partly compensated by the livelier acid decomposition in the young leaves than in the old. Thus in the

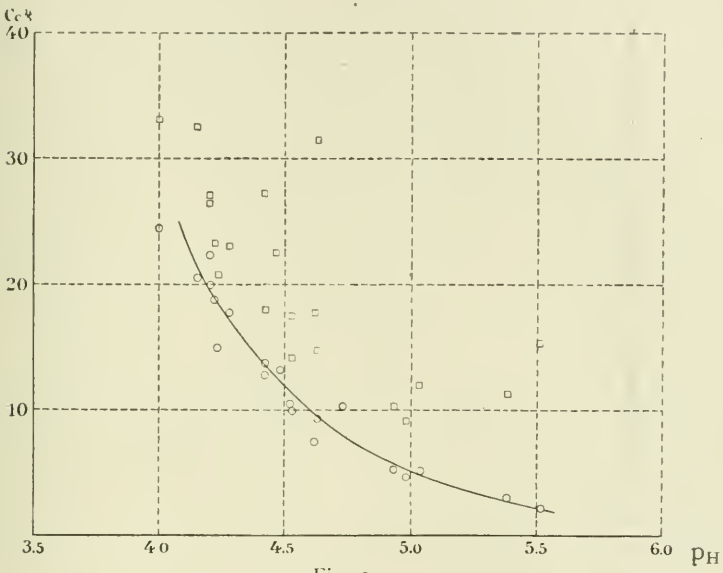

Fig. 2.

case of the first, a reduction of the quantity of acid corresponding to 9.29 cc. $^{n}/_{5}$ acid took place within the period named, whereas the loss in the case of the latter amounted only to 4.28, or less than half. That the acid decomposition should proceed so slowly in the old leaves cannot be explained as due to paucity of acid content, since Exp. 3 shows that the quantity of acid may be considerably further reduced. Moreover, the difference in quantity of acid between a and b is sufficient indication of a far more intense metabolism in the young leaves.

3. COTYLEDON CORUSCANS Haw.

The cell-sap was easily cleared of chlorophyll except in the first portion; this will in all probably account for the high nitrogen

COTYLEDON CORUSCANS Haw.

Table 3.

	g. leaves	% dry matter	% water	pH Lakmoïd-test		pH Electrom. measurement		Direct titration values		Litmus cc. n/5 per 100 g. sap	Phenolpht. cc. n/5 per 100 g. sap	Phenolpht. ÷ Litmus	mg. N per 100 g. sap
				before	after	Hasselbalch's method	H-current method	Litmus cc. n/5	Phenolpht. cc. n/5				
1. Left in light; examined 5 June 1912 1.45	50.26	3.53	96.47	4.5	4.5	4.33	—	8.89	13.94	31.64	49.61	17.97	12.10
2. 24 hours in darkness; examined 6 June 1912 1.45	46.76	2.60	97.40	3.9	3.9	4.11	—	12.98	14.64	45.26	51.05	5.79	4.84
3. Left in light; examined 29 Sept. 1913 11.15	34.84	2.39	97.61	4.3	—	4.27	4.30	5.97	6.71	34.37	38.63	4.26	—
4. 19½ hours in darkness; examined 30 Sept. 1913 10.45	39.42	2.10	97.90	4.0	4.0	4.01	4.07	10.98	11.88	46.02	49.79	3.77	—
5. Left in sunlight; exam. 30 Sept. 1913 3.20	57.40	2.41	97.59	5.6	—	—	5.43	1.46	2.06	5.05	7.13	2.08	7.28

content therein found. In this portion, also, titration to the phenolphtaleïn point proved not a little difficult, and somewhat uncertain, whereas in the remaining portions it went off easily enough. The values found for p_H varied between 4 and 5.5, i. e.

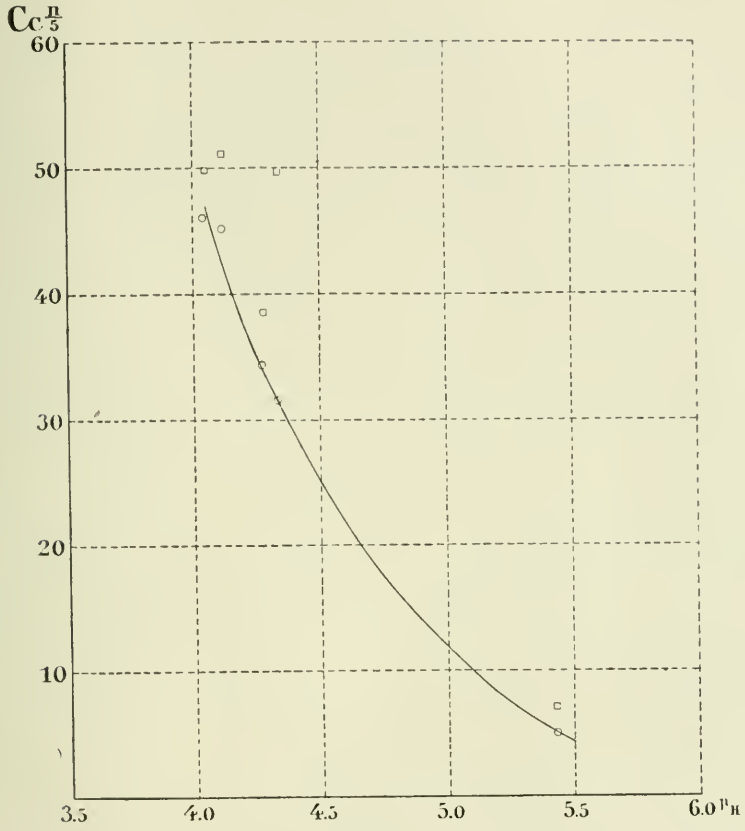

Fig. 3.

within the same range as found in the case of the species previously dealt with; as to how far a greater rise or fall of the hydrogen ion concentration in this plant may be possible I will not venture to say. In any case, it is not easy to imagine any essential advance in the alkaline direction, since the acid value corresponding to lowest concentration of hydrogen ions was very low, to wit: 5.05, in comparison with the highest found, which was 46.02. The difference between phenolphtaleïn and litmus figures was found to be very slight in all cases save the first, where the juice proved very difficult to cleanse; there is there-

fore some probability that part of the "impurities" found in this portion of the juice may have played the part of weak acids. Possibly also, this plant may have been older, or in other respects unlike the rest.

4. COTYLEDON OBVALLATA Haw.

The experiments with this plant were carried out at very different times. In Exp. 8 a and b, Table 4 a, young and old leaves are kept separate, the first portion consisting of leaves from the upper part of the stem, which was still in full growth, while those used for portion b were taken from the lower parts of the stem, and had almost, if not entirely, attained their full size and developement. In the remaining portions no such distinction was made. For Exp. 1, 2, 3 and 5, Table 4 a, large old plants were used; also for Exp. 1—6 in Table 4 b an old plant was used (the same specimen for all experiments) the leaves from this being so selected as to keep the portions as nearly as possible alike with regard to age and size of the leaves. For the remaining experiments, fairly young plants were employed; within each of the experiments 4, 6, 7 and 8, Table 4 a, and 7—9 Table 4 b the portions were kept as nearly uniform as possible.

Exp. 4, 5 and 6 (Table 4 a) for which the temperatures are shown in the curves 3 and 4, reveal the effect of almost equally long periods of darkness on the acid metabolism; in 4 and 5, the quantity of acid has distinctly increased during the 21—25 hours of darkness, whereas the quantity of acid in 6 a, in which it had been considerably higher than in the corresponding portions 4 a and 5 a, instead of increasing, actually decreased during the 24 hours' darkness, and this despite the fact that the temperature for the three dark periods was very nearly the same, as will be seen from the temperature curves. We have here, then, an excellent instance of manner in which plants may be differently disposed at different times.

From Exp. 7, Table 4 a, it will be seen how great is the influence of light upon the decomposition of acid, this factor having here reduced the quantity of acid from c. 13 to 4 cc. $^n/_5$ in the course of some 4 hours, despite the fact that the plant was not particularly rich in acid, and had been previously exposed to light.

TABLE 4a.

	g. leaves	% dry matter	% water	pH Lakmoïd test		pH Electrom-measurement		Direct titration values		Litmus cc. n/5 per 100 g. sap	Phenolpht. cc. n/5 per 100 g. sap	Phenolpht. ÷ Litmus	mg. N per 100 g. sap
				before	after	Hasselbalch's method	H-current method	Litmus cc. n/5	Phenolpht. cc. n/5				
1. Stood in south window of a room at 18-20°; exam. 4 Dec. 1912	60.92	1.75	98.25	5.1	—	5.03	5.09	1.05	1.87	4.68	8.33	3.65	3.49
2. Left in light in glasshouse; examined 26 May 1913	132.40	—	—	5.0 4.9	—	—	4.81	0.98	1.73	6.73	11.87	5.14	—
3 a. Left in light; examined 27 May 1913	40.18	—	—	5.4	—	—	5.46	0.67	2.25	2.72	9.14	6.42	—
3 b. Leaves picked same time as a; 48 hours in darkness at 8-12°; examined 29 May	40.76	—	—	4.8	—	—	4.76	1.81	3.11	8.27	14.21	5.94	—
4 a. Plant left in sunlight; examined 26 August 1913 1.45	95.17	2.38	97.62	4.9	—	—	4.77	2.04	3.17	8.16	12 68	4.52	4.37
4 b. As a; thereafter 25 hours in darkness; examined 27 August 2.45	75.92	2.35	97.65	4.6	—	4.34	4.39	3.78	5.20	15.12	20.80	5.68	4.93
5 a. Left in sunlight; examined 29 August 1913 3 o'c.	136.54	1.83	98.17	4.8	—	4.39	4.35	3.27	4.21	13.08	16.84	3.76	2.94
5 b. As a; thereafter 21½ hours in darkness; exam. 30 August 12.30	128.95	1 68	98.32	4.3	4.3	4.16	4.04	4.67	5.55	18.68	22.20	3.52	2.98
6 a. Left in light; examined 1 Sept. 1913 1 o'c.	69.18	1.68	98.32	4.5	4.5	4.22	4.26	5.47	6.29	21.88	25.16	3.28	3.30
6 b. As a; thereafter 24 hours in darkness; examined 2 Sept. 1 o'c.	59.18	1.90	98.10	4.6	4.6	4.35	4.32	4.39	5.69	17.56	22.76	5.20	4 17
7 a. Plant left in diffused daylight; examined 6 Sept. 1913 11 o'c.	63.86	1.85	98.15	4.6	4.6	4.47	4.49	3.21	5.05	12.84	20.20	7.36	4.29
7 b. As a; thereafter 4⅛ hours in sunlight; exam. 6 Sept. 3.20	74.33	1.90	98.10	5.4	5.3	5.06	5.10	1.02	2.28	4.08	9.12	5.04	5.08
8 a. Left in light; examined 8 Sept. 1913 10.30 — Young leaves (sixteen)	22.36	2.34	97.66	4.6	4.7	(4.41)	4.44	1.94	3.78	13.29	25 89	12.60	8.44
8 b. 8 Sept. 1913 10.30 — Older leaves (nine)	39.22	1.89	98.11	4.9	4.9	4.74	4.74	1.85	3.33	7.40	13.32	5.92	4.81

	g. leaves	% dry matter	% water	Electrom. measurement		Direct titration values	
				Hasselbalch's method	H-current method	Litmus cc. n/5	Phenolpht. cc. n/5
1. Left in light; examined 23 Sept. 1914 3 o'c.	106.17	1.48	98.52	(4.88)	4.91	1.10	2.17
2. Left 3 1/2 hours in darkness; examined 24 Sept. 1914 3.10	98.62	1.47	98.53	(4.66)	4.68	1.32	2.13
3. Left in light; examined 25 Sept. 1914 3.30	112.26	1.55	98.45	5.29	5.31	0.58	1.28
4. Left 2 hours in darkness; examined 29 Sept. 1914 2.15	108.88	1.43	98.57	4.89	4.94	1.48	2.41
5. 6 1/2 hours in darkness; examined 30 Sept. 1914 3.40	103.12	1.54	98.46	4.44	4.51	2.68	3.70
6. 6 3/4 hours in darkness; examined 2 October 1914	64.84	1.27	98.73	4.58	4.66	0.83	1.09
7. Left 4 hours in darkness; examined 21 Nov 1914	148.35	1.84	98.16	4.89	4.92	1.03	2.27
8. Left in light; examined 23 November 1914	110.44	2.08	97.92	5.08	5.10	0.90	2.07
9. Plant left in light; examined in November 1914	—	—	—	—	—	—	—
Mean values ..	—	—	—	—	—	—	—

In the case of this plant, determinations of ash were made in the experiments noted in Table 4 b. A fairly high degree of constancy is observed as regards the different ash components, so long as we keep to one and the same plant, but they appear to vary more between different plants. Thus in the first six experiments, all made with one and the same plant, 9.5—11 mg. equivalent calcium was found, against 7—8 mg. equivalent in the three last. And as regards the magnium, the juice of the first plant showed slightly over 1 mg. equivalent, whereas in the last, only 1/2 mg. equivalent, or less, was found.

In the six first experiments, no determination of metallic alkalis was made, and in five of them (Nos. 2—6) aluminium and iron were also disregarded. In these experiments, as already mentioned, the ash is heated at a glow to constant weight, which is noted in the column marked ash. This value will not include

	per 100 g. sap													
Phenolpht. cc. n/5	Phenolpht ÷ Litmus	mg. N	SiO_2	$Al_2O_3 + Fe_2O_3$		CaO		$Mg_2P_2O_7$		$K_2SO_4 + Na_2SO_4$		Ash g.	MgO g.
				g.	mg. equivalent	g.	mg. equivalent	g.	mg. equivalent	g.	mg. equivalent		
8.69	4.28	5.25	0.0018	0.0294	1.73	0.2630	9.38	0.0623	1.12	—	—	0.3172	0.0226
10.40	3.95	6.15	0.0004	(0.0300)	(1.76)	0.2668	9.51	0 0661	1.19	—	—	0.3207	0.0239
5.37	2.94	4.70	0.0019	(0.0287)	(1.69)	0.3012	10.74	0.0659	1.18	—	—	0.3538	0.0239
7.32	1.98	3.68	—	(0.0160)	(0.94)	0.3074	10.98	0.0654	1.17	—	—	0 3471	0.0237
12.33	3 40	6.17	—	(0.0275)	(1.61)	0.3126	11.14	0.0681	1.22	—	—	0.3648	0.0247
10.46	2.49	4.89	—	(0.0330)	(1.94)	0.2966	10.57	0.0651	1.17	—	—	0.3532	0.0236
11.26	6.15	1.49	—	0.0698	4.10	0.1992	7.10	0.0318	0.57	0.1807	2.07	—	—
10.24	5.79	2.72	—	0.0752	4.42	0.2015	7.18	0.0215	0.39	0.1960	2.25	—	—
—	—	—	—	0.1013	5.95	0.2342	8.35	0.0025	0.04	0.1733	1.99	—	—
—	—	—	—	—	—	—	9.44	—	0.89	—	2.10	—	—

metallic alkalis, these having, at least in part, been burnt away; it will, however, serve as a measure for the total quantity of the remaining components. By transposing the values found for $Mg_2P_2O_7$ to MgO, we obtain the values noted under MgO, and by subtracting these and the corresponding CaO values from the quantity of ash, we obtain values which may doubtless be taken, without serious error, as corresponding to the amount of $Al_2O_3 + Fe_2O_3$; the quantities thus calculated are here shown in parentheses (in 2—6). How great the quantity of metallic alkalis in the six first experiments may have been it is of course impossible to say; everything points, however, to the conclusion that it can have been but small. In the first place, only c. 2 mg. equivalents were found in 7—9, and in the second, the "ash weight" became constant at a comparatively early stage of the heating. Thus in the case of this plant, constant weight was

attained after c. 4 hours' heating, whereas the ash of Rochea falcata *(vide infra)* which contains far greater quantities of alkalis, had after 14—15 hours' heating still not reached constant weight.

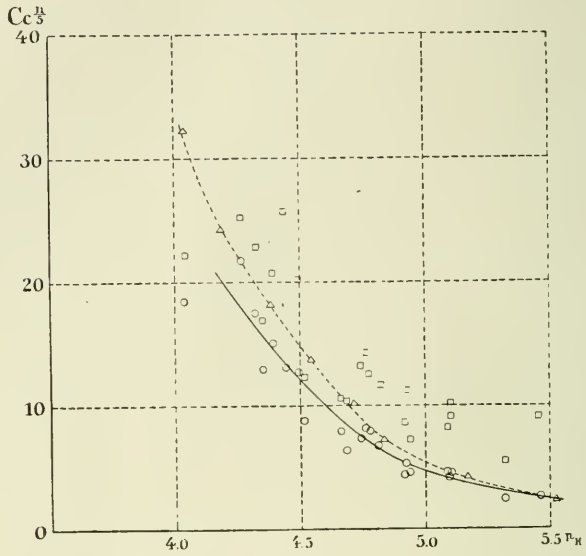

Fig. 4.

5. CRASSULA OBOVATA Haw.

Only a small amount of material from this species was available, and only a few experiments were thus made; the results will, however, be of value when taken in conjunction with those for the other succulents.

It will be seen from Table 5 that this plant resembles the remaining succulents examined, as is also evident from the curve in Fig. 5. Here, as in the case of Cotyledon coruscans, we are justified in drawing up curves based on so few analyses by the fact that these plants exhibit so great a degree of similarity to the others concerned.

6. CRASSULA LACTEA Soland.

In Exp. 1 a, b, and 2 a, b, strong, old plants were used. For No. 3, on the other hand, 5 quite young plants, all alike, were used; from four of these, every alternate leaf was taken for 3 a,

CRASSULA OBOVATA Haw.

TABLE 5.

	g. leaves	% dry matter	% water	pH Lakmoïd test		pH Electrom. measurement	Direct titration values		Litmus cc. n/5 per 100 g. sap	Phenolpht. cc. n/5 per 100 g. sap	Phenolpht. ÷ Litmus	mg. N per 100 g. sap
				before	after	Hasselbalch's method	Litmus cc. n/5	Phenolpht. cc. n/5				
1. Left in light; examined 8 May 1912 1.30	39.76	3.71	96.29	5.2	—	—	1.24	2.92	10.53	25.04	14.51	2.57
2. As 1; thereafter 24 hours in darkness; examined 9 May 1912	53.93	3.40	96.60	4.1	4.1	3.96	12.94	17.61	46.16	62.82	16 66	3.44
3. Left in light; examined 22 August 1912 2 o'c.	66.85	3.41	96.59	4.6	4.6	4.31	5.80	8.90	29.00	44.50	15.50	3 40
4. 48 hours in darkness; examined 7 Sept. 1912	55.57	3.71	96.29	4.3	4.3	4.24	11.16	15.35	40.07	55.12	15.05	3.99

the remainder being devoted to 3 b. For 3 c, the whole of the fifth plant was used. On the 17th October, the temperature at 4 o'clock was 19°; in the course of the evening and following

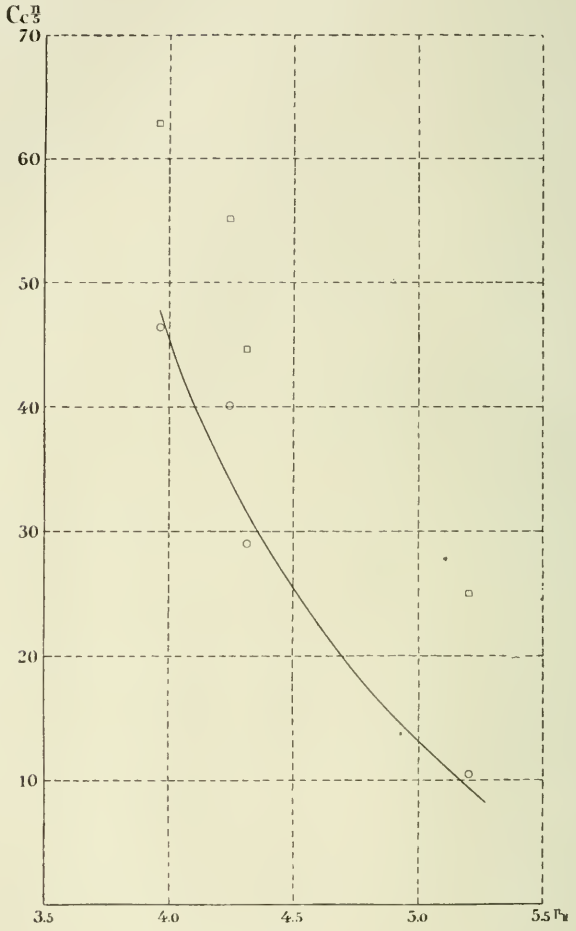

Fig. 5.

night it fell to 9°, rising again the next forenoon to 20—22½°, and remaining at this from noon till four o'clock.

Exp. 3 a and b give some idea as to the intensity with which the acid decomposition can take place, a light exposure of only some four hours having here brought a previously considerable quantity of acid (47.24 cc. n/5) down to a comparatively small amount (14.71 cc. n/5).

TABLE 6.

	g. leaves	% dry matter	% water	pH Lakmoïd-test before	pH Lakmoïd-test after	pH Electrom. measurement Hasselbalch's method	pH Electrom. measurement H-current method	Direct titration values Litmus cc. n/5	Direct titration values Phenolpht. cc. n/5	Litmus cc. n/5 per 100 g. sap	Phenolpht. cc. n/5 per 100 g. sap	Phenolpht. ÷ Litmus	mg. N per 100 g. sap
1a. Left in light; examined 15 August 1912 2 o'c.	116.17	5.17	94.83	4.4	4.4	4.43	—	13.97	27.48	28.39	55.85	27.46	4.35
1b. As a; thereafter 23½ hours in darkness; examined 16 August 1.30	106.93	5.25	94 75	4 3	4.3	4.33	—	13.50	26.86	31.84	63.35	31.51	5.00
2a. Left in light; examined 12 Sept. 1912 2.45	88.33	5.55	94.45	5.2	5.2	5.13	—	2.26	10.34	7.53	34.47	26.94	3 77
2b. 48 hours in darkness; examined 14 September	61.37	5.27	94.73	4.5	4.5	4 36	—	7.02	14.35	28.48	58.22	29.74	5.19
3a. 19 hours in darkness; examined 18 Oct. 1913 11.15	41.06	2.56	97.44	3.95	3.95	3.96	4.00	13.67	15.36	47.24	53.08	5.84	5.32
3b. 19 hours in darkness; thereafter 4¼ hours in sunlight; examined 18 Oct. 3.30	38.64	2.75	97.25	4.8	4.8	4.62	4.69	3.89	5.04	14.71	19.06	4.35	6.63
3c. Had not been artificially darkened; examined 18 Oct. 2 o'c.	29.96	3.03	96.97	5.1	5.1	5.09	5.15	1.33	2.44	7.39	13.56	6.17	5.54

7. ROCHEA FALCATA D. C.

De Vries states, that this plant contains calcium malate[1]), and that it secretes malic acid during the night. Analysis of the cell-sap showed that it contained malic acid, possibly some slight

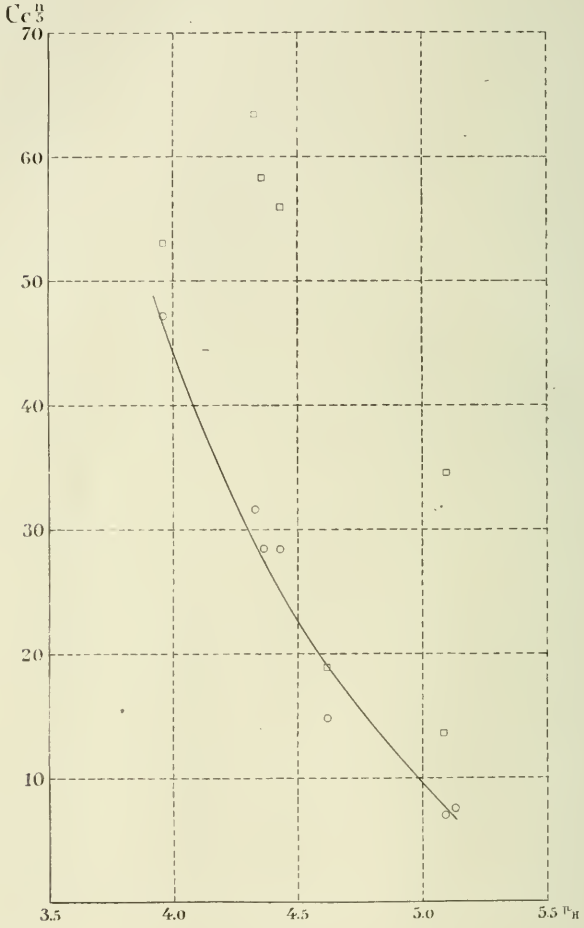

Fig. 6.

trace of citric acid, with traces also of hydrochloric acid, sulphuric and phosphoric acid.

With regard to the experiments, all results of which are shown in Table 7 a and 7 b, the following should be noted: For

[1]) De Vries: Bot. Zeitung **42**, 337 ff. (1884).

1 a and b, Table 7 a, the same plant was used, the leaves being so selected with regard to age and position as to render the two portions as far as possible alike; the same principle was adhered to in Exp. 3, 5, 7, 8 and 9, Table 7 a.

For Exp. 1—7, Table 7 b, three plants were used, the leaves being taken in such a manner as to keep all portions as nearly as possible alike; the same applies to the portions in 8. 9, 10 Table 7 b, for which two plants were used. All these five plants, which were from the same nursery, were very similar in respect of size, developement and age.

The plants used for 8 and 9 Table 7 a were much younger and smaller than those used in the foregoing experiments (1—6); they were very much alike in size and developement, had stood side by side in the glasshouse, and were treated alike throughout, save for the fact that 9 a was left $19^3/_4$ hours in darkness after 8 b had been taken out for analysis. It will therefore be permissible to compare the two portions 8 b and 9 a with 3 a and 3 b (Table 7 a), which latter show the influence of 24 hours' darkness upon the quantity of acid; this is, besides, the more justifiable in view of the fact that the temperature in the two periods in question (19 August 1912 4 o'c. to 20 August 2 o'c. for Exp. 3, and 15 September 1912 4 o'c. to 16 September 11.45 for 8 b, 9 a) was practically the same (*vide* curves 1 and 5). The difference between the litmus figures was for 3 a and b 9.56 cc. $^n/_5$, and for 9 a and 8 b 20.27 cc. $^n/_5$; i. e. the acid secretion twice as great in the younger plant. True, it may be objected that 8 b had a lower acid content than 3 a (6.46 as against 20.16 cc. $^n/_5$) and it would therefore be justifiable, on the principle that accumulation of matter in the cells generally impedes the further developement of the latter, to expect, *cæteris paribus*, a greater secretion of acid here than in the last case. The objection, however, does not hold good, as the acid content can rise to values far above those here noted; in 1 a and b, for instance, we have 43.84 and 59 79 cc. $^n/_5$ with an increase of 15.95 cc. $^n/_5$ acid during a period of 24 hours' darkness. (In this time, the temperature varied from c. 16^0 to 10^0 during the afternoon and night, rising again next forenoon to 28^0). No. 7 Table 7 a likewise shows the increase of acid during 24 hours of darkness; here the amount of acid increased by a quantity corresponding to 8.52 cc. $^n/_5$; a value even lower than that found in Exp. 3. This

	g. leaves	% dry matter	% water	pH Lakmoïd-test	
				before	after
1 a. Left in light; examined 3 June 1912 2.30	63.00	6.23	93.77	4.3	4.3
1 b. 24 hours in darkness; examined 4 June 2.30	61.27	5.62	94.38	4.05	4.0
2. Left in light; examined 10 June 1912 3 o'c.	16.67	6.86	93.14	5.5	5.5
3 a. Left in light; examined 19 Aug. 1912 2 o'c.	109.97	6.53	93.47	4.7	4.7
3 b. 24 hours in darkness; examined 20. Aug. 2 o'c.	122.00	6.78	93.22	4.4	4.4
4. 72 hours in darkness; examined 24 Aug. 1912 2 o'c.	98.03	7.35	92.65	4.3	4.3
5 a. Left in light; examined 10 Oct. 1912 2.30	90.04	8.78	91.22	4.6	4.6
5 b. 48 hours in darkness; examined 12 Oct. 1912 2.45	74.00	8.51	91.49	4.5	—
6 a. Left in strong sunlight; examined 14 June 1913 3 o'c.	45.19	6.52	93.48	—	—
6 b. Leaves picked same time as a; examined 16 June 11 o'c.	54.90	6.17	93.83	—	—
6 c. Leaves picked same time as a; examined 16 June 7.30	64.65	6.32	93.68	—	—
6 d. Leaves picked same time as a; examined 17 June 12 o'c.	53.19	5.77	94.23	—	—
7 a. Left in light; examined 8 July 1913 1.45	102.14	8.35	91.65	4.8	—
7 b. 25 hours in darkness; examined 9 July 2.45	110.73	6.54	93.46	—	—
8 a. Left in strong sunlight; examined 15 Sept. 1913 12.40	69.33	4.10	95.90	4.9	—
8 b. Left further $3\frac{1}{4}$ hours in sunlight; examined 15 Sept. 4 o'c.	69.11	4.21	95.79	5.4	—
9 a. $19\frac{3}{4}$ hours in darkness; examined 16 Sept. 1913 11.45	46.23	4.00	96.00	—	—
9 b. $19\frac{3}{4}$ hours in darkness; thereafter $3\frac{1}{4}$ hours in light (weather dull); exam. 16 Sept. 3 o'c.	47.91	4.49	95.51	—	—

TA D. C.

Electrom. measurement		Direct titration values		Litmus cc. $^{n}/_{5}$ per 100 g. sap	Phenolpht. cc. $^{n}/_{5}$ per 100 g. sap	Phenolpht. ÷ Litmus	mg. N per 100 g. sap
el-'s od	H-current method	Litmus cc. $^{n}/_{5}$	Phenolpht. cc. $^{n}/_{5}$				
4	—	14.03	25.01	43.84	78.16	34.32	14.7
5	—	11.42	18.44	59.79	96.54	36.75	21.2
8	—	0.50	3.60	6.20	44.67	38.47	—
4	—	5.04	12.48	20.16	49.92	29.76	3.80
6	—	7.43	13.73	29.72	54.92	25.20	4.32
3	—	10.10	18.88	33.67	62.93	29.26	—
	4.67	4.32	15.09	19.82	69.22	49.40	5.23
	4.29	8.02	18.87	34.87	82.04	47.17	5.08
	5.39	1.11	10.60	5.49	52.45	46.96	—
	4.75	4.61	16.02	17.18	59.71	42.53	—
	5.03	3.84	12.59	13.16	43 15	29.99	—
	4.80	3.58	11.67	14.19	46.25	32.06	—
	4.71	8.63	33.41	22.79	88.25	65.46	—
	4.34	7.10	16.83	31.31	74.21	42.90	6.42
6	4.81	3.24	9.50	12.96	38.00	25.04	31.44
7	5.20	1.48	6.44	6.46	28.12	21.66	30.28
7	4.30	6.33	12.70	26.73	53.63	26.90	24.91
8	4.81	2.95	8.26	12.82	35.88	23.07	33.67

	g. leaves	% dry matter	% water	pH Electrom. measurement: Hasselbalch's method	pH Electrom. measurement: H-current method	Direct titration values: Litmus cc. n/5	Direct titration values: Phenolpht.
1. Left in light; examined 6 Oct. 1914	137.70	6.57	93.43	4.46	4.48	6.27	11.1
2. Left 24 hours in darkness; examined 7 Oct. 1914	171.89	5.83	94.17	4.10	4.11	12.36	16.9
3. Left in light; examined 9 Oct. 1914	132.26	7.26	92.74	4.32	4.33	6.73	11.1
4. 21½ hours in darkness; exam. 10 Oct. 1914	167.35	7.11	92.89	4.14	4.16	9.64	15.1
5. Left in light; examined 12 Oct. 1914	134.16	5.92	94.08	4.30	4.30	8.03	12.8
6. Left 24 hours in darkness; examined 13 Oct. 1914	133.68	6.82	93.18	4.24	4.25	6.79	11.1
7. Left in light; examined 14 Oct. 1914	122.72	4.27	95.73	(4.58)	4.57	4.67	7.3
8. Left in light; examined 21 Oct. 1914	122.51	4.90	95.10	4.46	4.46	4.76	7.1
9. Left in light; examined 23 Oct. 1914	130.83	4.18	95.82	4.23	4.23	6.89	9.!
10. Left in electric light for 5 hours; examined 28 Oct. 1914	138.81	4.55	95.45	4.72	4.73	2.83	5.(
Mean values...	—	—	—	—	—	—	–

will then serve well to support the theory that acid secretion takes place with less intensity in old leaves, but it cannot altogether be taken without reserve, as the temperature during the dark period was very high (17—38°, *vide* temperature curve 2), which, as we know, goes far to advance the process of acid decomposition.

For Exp. 6 Table 7 a, all leaves were picked off together on the 14th June, at 3 o'clock, and one portion (a) examined at once. The remainder were placed in a respiratory apparatus, through which a current of air without carbonic acid was constantly passing. On the 16th June at 11 o'clock, i. e. after a lapse of 43 hours, during which time the temperature had been maintained at 17° by means of a water bath, and the leaves etc.

	per 100 g. sap											
	Phenolphtalein cc. n/5	Phenolpht. + Litmus	mg. N	SiO_2	$Al_2O_3 + Fe_2O_3$		CaO		$Mg_2P_2O_7$		$K_2SO_4 + Na_2SO_4$	
					g.	mg. equivalent	g.	mg. equivalent	g.	mg. equivalent	g.	mg. equivalent
9	48.30	21.01	12.67	—	—	—	—	—	—	—	—	—
5	63.77	17.22	12.20	0.0012	0.0479	2 81	0.2042	7.28	0.0751	1.35	—	—
3	56.66	22.43	11.85	—	—	—	—	—	—	—	—	—
6	65.97	23.91	10.57	—	0.0803	4.71	0.2543	9.07	0.0907	1.63	0.5336	6.12
2	54.79	20.47	10.27	—	—	—	—	—	—	—	—	—
8	58.31	22.83	8.89	—	0.0728	4.27	0.2263	8.07	0.0782	1.40	0.5604	6.43
5	33.03	11.98	8.03	—	0.1129	6.63	0.2064	7.36	0.0615	1.10	0.5633	6.46
1	37.45	12.34	10.92	0.0025	0.0850	4.99	0.2564	9.13	0.0961	1.73	0.5867	6.73
2	46.14	12.92	5.02	—	0.0678	3.98	0.2512	8.96	0.0554	0.99	0-4520	5.18
4	28.64	12.50	4.96	—	0.0686	4.03	0.2720	9.70	0.0479	0.86	0.4934	5.66
	—	—	—	—	—	4.49	—	8.51	—	1.29	—	6.10

kept in the dark, a sample (b) of the leaves was taken out and examined, the remainder being left in the respiratory apparatus, which was now placed in strong sunlight, at ordinary indoor temperature. At 7.30 on the evening of the same day, i. e. after a lapse of $8^1/_2$ hours, another sample (c) was taken and analysed, the apparatus with the rest of the leaves being then darkened and placed in a water bath at 10^0, remaining thus until the next day at noon ($16^1/_2$ hours), when the last of the leaves (portion d) were subjected to analysis.

The respiratory apparatus consisted of a retort containing the material, with a close-fitting rubber stopper, bored to receive two glass tubes, one short and one long; through the latter a current of air free from carbonic acid was directed, this having

first passed through a tube containing toluol water and thereafter a tube with distilled water to saturate the air with moisture. The air was passed off from the retort by suction along the short glass tube, through two retorts containing potassium hydroxide in order to absorb the carbonic acid given off by the leaves. The results were as follows:

b.	c.	d.
19.67 mg.	93.23 mg.	2.40 mg. CO_2

the carbonic acid given off being calculated as per 100 g. leaves per 24 hours. This experiment is of no interest from the point of view of respiration; it shows, however, that secretion or decomposition of acid may take place in isolated leaves, and further, reveals the cause of the slight secretion of acid during the previous 16 hours' darkness. Taking into consideration the fact that the leaves had only been exposed to light for $8^1/_2$ hours of the previous 48, and had, moreover, during that time been subjected to a current of air without carbonic acid, it will be reasonable to suppose that the isolated leaves were gradually reduced to a state of starvation, whereby the material for acid secretion was, if not exhausted, at least greatly reduced.

As in the case of Cotyledon linguæfolia, so also here it would seem as if the acid metabolism takes place with greater intensity in young leaves than in older ones, which is, as noted on p. —, also indicated by comparison of 3 a and b with 8 b and 9 a in Table 8 a.

8. KLEINIA (CUNEIFOLIA D. C.?).

Experiments with Kleinia were carried out at very different times. As a rule, the cell-sap presented considerable difficulty in connection with the p_H determinations; only in two cases (4 b and 5 a) were certain results obtained by Hasselbalch's method; in the remaining cases, different values were obtained each time the liquid was renewed in the electrode tank, and as a rule in such a manner that the concentration of hydrogen ions fell. The hydrogen current method on the other hand, generally proved practicable; only in the case of the last two portions was it found impossible even in this way to arrive at certain results, the values varying now to one side, now to the other. These

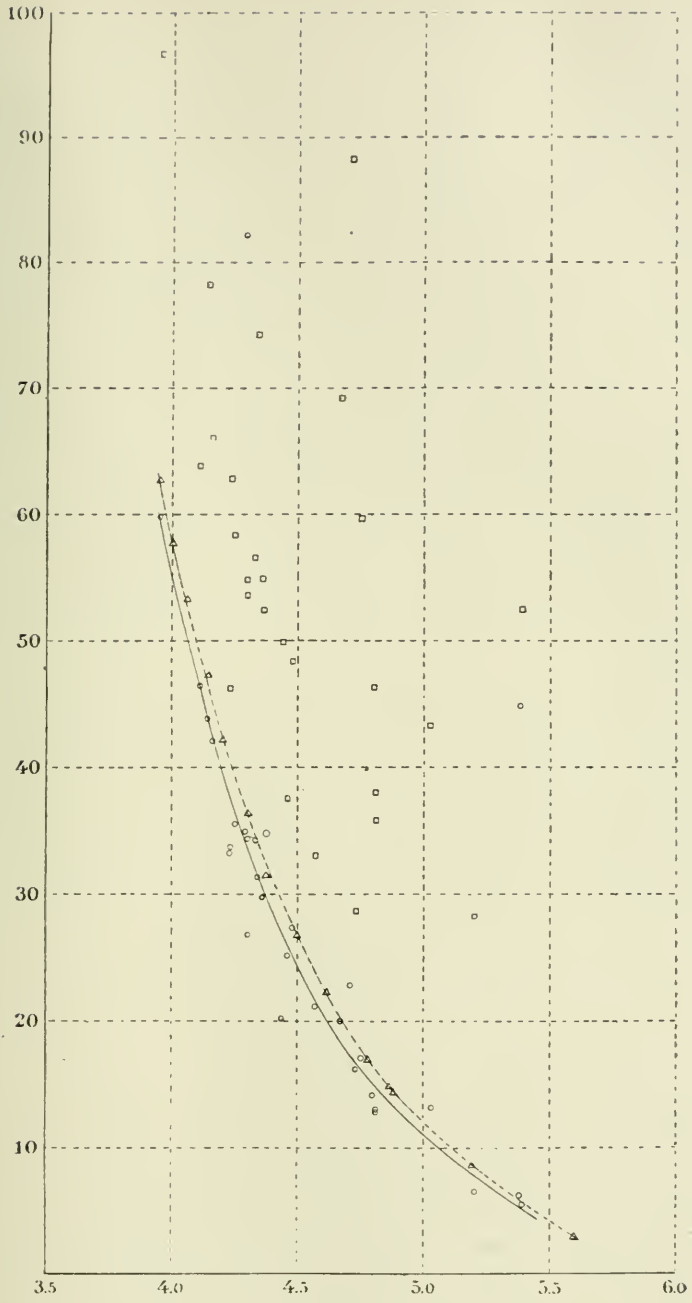

Fig. 7.

irregularities may perhaps be partly due to the fact that it was found quite impossible to clear the sap of chlorophyll.

Presumbly, some chemical processes take place during shaking in the grains of chlorophyll present in the liquid, the air naturally contained in the sap not being carried off, but merely mixed with the hydrogen originally enclosed in the electrode tank, while these processes cease on the transmission of hydrogen, the air from the liquid being then driven off by the hydrogen current.

The arrangement of the experiments will in most cases be evident from the tables; the plant used for Exp. 3 was kept under a large glass skylight at the top of the laboratory building[1]), where the temperature varied between 8 and 11°. The leaves for a and b were picked off simultaneously, the a portion being examined at once, those for b being left for twentyfour hours under a black shade beside the mother plant, on which remained the leaves used for c, and which were analysed simultaneously with b. In the course of these 24 hours, the picked leaves had lost 1.50 g. in weight (and weighed then 29.42 g.) and a corresponding weight of water was therefore added when crushing the leaves

For Exp. 7, four plants were used, the leaves being taken in such a manner as to let the two lots of small leaves, a and c, and the two lots of large, b and d, correspond as nearly as possible. The small leaves varied in length from 4 cm. to a few mm., while the large ranged from 8 to 4 cm. By this means it was possible to compare directly the acid metabolism in quite young leaves with that in those more developed. With regard to concentration of hydrogen ions, there appears to be no essential difference between young leaves and old; the determinations are, however, in the two last cases, uncertain. The litmus titrations show that the 4 hours' exposure to the sun occasioned in the older leaves a loss of acid answering to 25.94 cc. $^n/_5$ acid per 100 g. of sap, or abt. 83 % of the original quantity, whereas the corresponding amount in the small leaves answers to 20.15 cc. $^n/_5$ per 100 g. sap, i. e. abt. 69 % of the original amount. Whether this represents a real difference in the intensity of the metabolism, or whether the difference indicated should be explained as exclusively due to individual variation, I do not

[1]) In the remaining experiments the plants were kept in a glasshouse.

TABLE 8.

	g. leaves	% dry matter	% water	pH Lakmoïd-test: before	pH Lakmoïd-test: after	pH Electrom. measurement: Hasselbalch's method	pH Electrom. measurement: H-current method	Direct titration values: Litmus cc. n/5	Direct titration values: Phenolpht. cc. n/5	Litmus cc. n/5 per 100 g. sap	Phenolpht. cc. n/5 per 100 g. sap	Phenolpht. ÷ Litmus	mg. N per 100 g. sap
1. Left in light; examined 3 Sept. 1912 2 o'c.	46.04	3.80	96.20	5.5	5.6	(5.35)	—	1.44	6.34	4.93	21.73	16.80	12 62
2. 48 hours (7—9 Sept.) in darkness; examined 9 Sept. 2.30	38 32	3.79	96.21	4.5	4 5	(4.34)	—	7.12	10.44	38.53	56.49	17.96	—
3a. Left in light; examined 28 November 1912	30.84	—	—	4.9	—	(4.62)	4.72	5.10	7 96	28.59	44.62	16.03	11.60
3b. Leaves cut off 28 November and left 24 hours in darkness before analysis	30.92	—	—	4.8	—	(4.63)	4.77	2.64	4.64	24.86	43.80	19.03	15.50
3c. 24 hours in darkness; leaves picked off and analysed 29 November	30.45	—	—	4.8	—	(4.65)	4.70	3.17	5.04	29.00	46.11	17.11	17.88
4a. Left in light; examined 12 July 1913 4.30	64.68	5.82	94.18	5.4	5.5	—	5.29	3.14	9.02	10.59	30.43	19.84	—
4b. 17 hours in darkness; examined 13 July 1913 12.30	98.13	4.18	95.82	4.4	4.4	4.28	4.36	8.60	11.33	43.00	56.65	13.65	—
5a. Left in strong sunlight; examined 15 Sept 1913 12.40	37.02	2.96	97.04	4.8	—	4.68	4.71	4.36	5.68	22.90	29.83	6.93	10.52
5b. Further 3¼ hours in sunlight; examined 15 Sept. 40'c.	40.94	2.85	97.15	5.8	—	—	5.55	0.58	1.70	2.89	8.47	5.58	7.94
6a. 20 hours in darkness; examined 16 Sept. 1913 11.45	43.38	2.38	97.62	—	—	—	3.97	13.31	14.20	62.28	66.45	4.17	6.34
6b. 20 hours in darkness; thereafter 3¼ hours in diffused daylight; examined 16 Sept. 3 o'c.	64.14	2.37	97.63	—	—	—	4.42	7.50	8.40	30.00	33 60	3.60	6.30
7a. Had been left in light; 7b. examined 18 Sept. 1913 11 o'c. — Small leaves	30.90	2.77	97.23	—	—	—	4.46	2.82	3 45	29.16	35.68	6 52	7.11
7b. — Large leaves	52.55	2 52	97.48	—	—	—	4.42	6.26	7.75	31.30	38.75	7.45	7 17
7c. Left further 4 hours in sunlight; 7d. examined 18 Sept. 3 o'c. — Small leaves	47.15	3.07	96.93	—	—	—	(c. 5.0)	1.97	2.04	9.01	13.45	4.44	5.42
7d. — Large leaves	87.07	2.57	97 43	—	—	—	(c. 5.1)	1.34	2.53	5.36	10.12	4.76	5.46

venture to say; the dissimilarity between the two values is, however, so considerable as to suggest a real difference.

The great variation in the litmus figures is also an expression of the very active metabolism in this species as regards

Fig. 8.

acid secretion and acid decomposition respectively. The experiment just mentioned shows how even an exposure to light of only 4 hours can cause the greater part (c. $^2/_3$ and $^5/_6$ respectively) of the acid present to disappear, although there had been no previous dark period. During the four hours the experiment lasted, the sun was shining, and the temperature varied between 23 and 27°, whereas at 6 in the morning it was 13°. Exp. 6

also showed a very considerable decomposition of acid; the plant in question had here been previously left in the dark, and had thus accumulated a considerable quantity of acid, the greatest amount ever found in any of the succulents examined. An exposure to light of only $3^1/_4$ hours (during which the sun was only shining for a short time about one o'clock, the weather for the rest of the time being dull and rainy) with a temperature varying between 18 and 27° (temp. curve No. 5) was sufficient to bring about a loss of acid equal to 32.28 cc. $^n/_5$ per 100 g. of sap. Similar marked indications of metabolism were found in 4 and 5. In Exp. 3 b and c quite different results were observed; the two portions of leaves had been left in the dark for the same length of time, the first lot, however, had been picked off, while the remainder were left still attached to the mother plant. In c, a very slight increase in the quantity of acid was noted, and a similarly slight increase in the concentration of hydrogen ions. Exp. b, on the other hand, showed a loss of acid, and corresponding reduction in the concentration of hydrogen ions. The explanation of this would seem to be, that the isolated leaves had partly met their respiratory consumption by decomposition of acid, whereas the leaves still on the stem could be supplied with matter from the remaining portions of the plant, so that the secretion and decomposition of acid could proceed at about the same rate.

The total values for quantity of nitrogen exhibit great variation, which in the case of this species can be explained simply by the chlorophyll contained in the sap.

Titration of the sap from this plant presented no particular difficulty; the green colour on the alkaline side of the litmus point grew very faint, while the phenolphtaleïn red was quite distinct.

9. MESEMBRIANTHEMUM ECHINATUM Lam.

For Exp. 1 a and b the same plant was used, the leaves being so selected as to keep the two portions as far as possible alike. For 4 a and b two plants were used; half the leaves from each plant were here taken for each portion, the leaves being so distributed as to keep a and b as nearly as possible alike. For Exp. 5, four plants were employed, one-third of the leaves from each being taken for each portion.

MESEMBRIANTHEMUM ECHINATUM Lam.

Table 9.

	g. leaves	% dry matter	% water	Lakmoïd-test		pH Electrom. measurement		Direct titration values		Litmus cc. n/5 per 100 g. sap	Phenolpht. cc. n/5 per 100 g. sap	Phenolpht. ÷ Litmus	mg. N per 100 g. sap
				before	after	Hasselbalch's method	H-current method	Litmus cc. n/5	Phenolpht. cc. n/5				
1a. Left in light; examined 22 April 1912 3 o'c.	24.37	2.77	97.23	—	—	5.68	—	0.09	1.25	0.58	8.10	7.52	—
1b. 22½ hours in darkness; examined 23 April 1.30	21.50	2.29	97.71	—	—	4.81	—	—	—	—	—	—	—
2. Plant left in strong sunlight; examined 26 Aug. 1912 2.30	77.47	2.17	97.83	5.2	5.5	5.06	—	2.60	3.81	10.40	15.24	—	10.76
3. Two plants left in sunlight; examined 22 Sept. 1913 11 o'c.	40.58	1.71	98.29	—	—	4.58	4.62	3.84	4.58	15.36	18.32	2.96	11.52
4a. 19 hours in darkness. Examined 23 Sept. 1913 11.30; thereafter	31.42	1.61	98.39	—	—	4.52	4.51	3.26	3.90	18.04	21.58	3.54	17.86
4b. 19 hours in darkness. 3½ hours in light; examined 23 Sept. 3 o'c.	47.16	1.49	98.51	—	—	4.88	4.81	2.69	3.50	10.76	14.00	3.24	20.52
5a. Left in light; examined 25 Sept. 1913 10.15	38.50	1.76	98.24	4.9	4.9	4.52	4.54	3.58	4.19	16.94	19.83	2.89	16.46
5b. Left further 4¼ hours in light; examined 25 Sept. 2.30	46.02	1.85	98.15	5.6	5.6	5.09	5.13	1.76	2.66	7.04	10.64	3.60	19.65
5c. Left further 22½ hours in darkness; examined 26 Sept. 1 o'c.	48.72	1.87	98.13	4.9	—	4.65	4.70	3.51	4.29	14.04	17.16	3.12	16.86

Exp. 4 shows a marked reduction of the quantity of acid during the $3^1/_2$ hours b was exposed to light, after a had been examined: the temperature rose during this period from abt. 17^0 to 23^0, as will be seen from temp. curve No. 6. A very similar decrease is noted for the quantity of acid in 5, where b shows litmus value 7.04 against the 16.94 for a; in this case, the loss of acid was produced in the course of $4^1/_2$ hours, during which time the temperature rose from 15 to 21^0. After being kept in

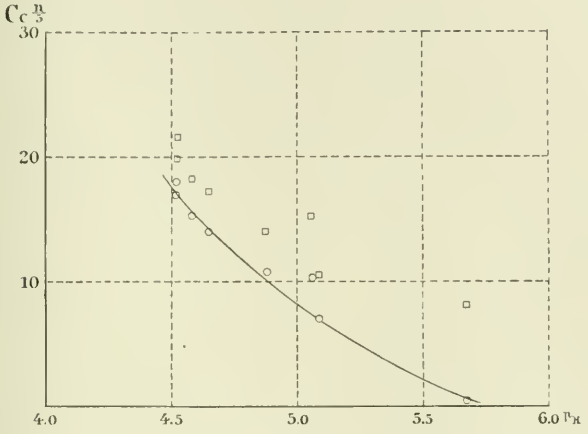

Fig. 9.

the dark, the plants again showed an increase of the acid content, albeit not reaching the value of a, which is somewhat remarkable, since the temperature during this dark period exhibited no great difference from that immediately preceding the examination of a, during which period, moreover, the plant had been exposed to the light. There must thus have been some reason for the fact that the acid secretion did not proceed with any great intensity, or possibly for the fact that a comparatively intense decomposition was taking place at the same time. This would be accounted for, if the leaves could be considered as having been in a kind of starvation condition, which is, however, hardly likely to have been the case, since the dark period was not of very great extent.

10. MESEMBRIANTHEMUM LINGAEFORME HAW.

According to the investigations made by Berg and C. Gerber[1]) the cell-sap of this plant contains mainly malic acid, with traces also of oxalic and citric acid.

The sap was in most cases easily cleared of chlorophyll by centrifuging and subsequent filtration, and was then of a light yellow colour, and nearly or entirely clear Only in Exp. 1 was it found impossible to eliminate the chlorophyll altogether, and in 6, the juice, after having been cleared of chlorophyll, was a muddy brown. The reason of this is unknown, the leaves were apparently quite fresh, and, as will be seen from the table, there is nothing to indicate that they were in any respect abnormal. Exp. 5, 6 and 7 were carried out in connection one with another. For each of these, one whole young plant was taken, all three plants being alike. Nos. 5 and 6 had been left standing in the sun, the latter $3^1/_2$ hours longer than the former, while No. 7 had further been kept for 20 hours in the dark. A decrease was also noticeable from 5 to 6 in the concentration of hydrogen ions, which had been fairly low to begin with, and an increase thereafter, with the 20 hours' darkness. On the other hand, these experiments showed not only an increase in the quantity of acid in the 20 hours' dark period, but also in the $3^1/_2$ hours exposure to light. It is hardly likely, however, that any real increase in the amount of acid should have taken place during this time; in all probability we have here an instance of marked individual variation, the material employed in 6 having been especially rich in acid. In all the experiments, the litmus and phenolphtaleïn figures are particularly low when compared with the corresponding values for other plants; this point will be further referred to later on. In addi-

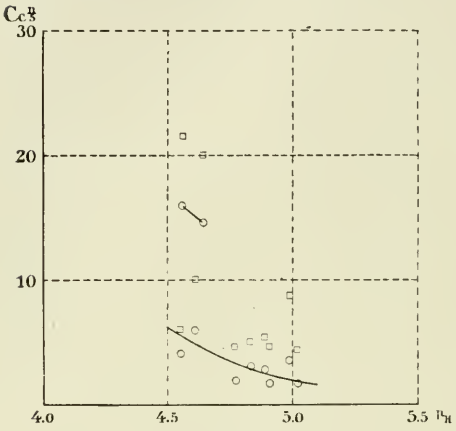

Fig. 10.

[1]) Revue générale de Botanique **8**, 295—302 (1896).

Table 10.

	g. leaves	% dry matter	% water	pH Lakmoïd-test		pH Electrom. measurement		Direct titration values		Lit-mus cc. n/5 per 100 g sap	Phe-nolpht. cc. n/5 per 100 g. sap	Phe-nolpht. ÷ Lit-mus	mg. N per 100 g. sap
				before	after	Hassel-balch's method	H-current method	Lit-mus cc. n/5	Phe-nolpht. cc. n/5				
1a. Left in light; examined 21 May 1912 2 o'c.	45.29	3.49	96.51	5.2	5.2	5.02	—	0.31	0.76	1.81	4.43	2.62	18.73
1b. 48.5 hours in darkness; examined 23 May 2.30	39.41	2.79	97.21	5.0	5.1	4.91	—	0.42	1.08	1.84	4.72	2.88	15.53
2a. Left in light; examined 3 Oct. 1912 2 o'c.	49.53	5.71	94.29	4.8	—	4.56	—	2.86	3.84	16.00	21.49	5.49	16.60
2b. 48.5 hours in darkness; examined 5 Oct. 2.30	65.05	4.81	95.19	4.9	—	4.64	—	3.01	4.12	14.70	20.13	5.43	18.35
3a. Left in light; examined 25 Sept. 1913 11 o'c.	37.46	3.47	96.53	5.2	5.2	4.77	4.84	0.37	0.83	2.08	4.66	2 58	22.05
3b. 26 hours in darkness; examined 26 Sept. 1 o'c.	26.00	2.56	97.44	4.9	—	—	4.55	0.42	0.60	4.20	6.00	1.80	17.73
4. Two small plants left in light; examined 27 Sept. 1913 2 o'c.	51.44	3.98	96.02	5.3	—	4.89	4.89	0.65	1.27	2.89	5.64	2.75	21.48
5. Left in sunlight; examined 29 Sept. 1913 12 o'c.	44.51	3.73	96.27	5.1	—	—	4.83	0.43	0.69	3.23	5.18	1.95	21.17
6. Left in sunlight; examined 29 Sept. 1913 3.30	36.08	3.77	96.23	5.4	—	—	4.99	0.30	0.70	3.75	8.75	5.00	15.44
7. Left 20 hours in darkness; examined 30 Sept. 1913 11 o'c.	39.59	3.43	96.57	4.8	4.8	—	4.61	1.25	2.12	5.95	10.09	4.14	9.05

tion, the difference between the mentioned figures is very slight compared with what we find in most of the other plants. No. 2 showed somewhat higher values for quantity of acid than noted in the remaining cases; on comparing the result with 7, which showed very nearly the same concentration of hydrogen ions, we find values 2—3 times as high as in the last case. It would seem reasonable to suppose that the material for Exp. 2 was derived from a different race, as I had procured the plant used for this at another time, independently of the remainder. This would also agree with the fact that the litmus points for 2 lie quite outside the range of the remaining litmus points.

11. MESEMBRIANTHEMUM LEHMANNII Eckl. & Zeyh.

The material of this plant was only sufficient for a single experiment.

The sap was, after centrifuging and filtration, quite green. Acid titration to phenolphtaleïn point was also a matter of considerable difficulty. The plant in question, of which only the leaves and uppermost, quite young and soft parts of the stem were used, was first examined on the 10 May at 1 o'clock, after exposure to light, and again 24 hours later, after being kept in darkness. The temperature on both days reached 19° C. about 1 o'clock, with minimum for the foregoing nights at 6° and 5° respectively.

MESEMBRIANTHEMUM LEHMANNI Eckl. & Zeyh.

Table 11.

	g. leaves	% dry matter	% water	pH Lak-moïd-test before	pH Lak-moïd-test after	pH Electrom. measurement Hasselbalch's method	Direct titration values: Litmus cc. n/5	Direct titration values: Phenolpht. cc. n/5	Litmus cc. n/5 per 100 g. sap	Phenolpht. cc. n/5 per 100 g. sap	Phenolpht. ÷ Litmus	mg. N per 100 g. sap
1. Left in light; exam. 10 May 1912 1 o'c.	66.21	6.25	93.75	5.6	5.7	5.35	0.92	3.04	3.00	9.92	6.92	57.84
2. 24 hours in darkness; examined 11 May 1 o'c.	57.47	6.35	93.65	5.0	5.1	4.81	2.02	3.76	7.70	14.38	6.68	54.62

The table shows very high figures for the nitrogenous com-

pounds in the sap, as is but natural in view of the chlorophyll content.

12. ALOE ARBORESCENS Mill.

Only a single experiment was made with this plant, owing to the paucity of material available; it is very difficult to work with, as the sap, which is very thick and slimy, almost gelatinous, can hardly be pressed from the leaves at all. When once extracted and centrifuged, moreover, it was found impossible to filter this juice, which was also of a very lumpy and irregular consistency.

The difference in p_H values and the values found by acid titration was only very slight. Whether we may conclude from this that the species in question never does exhibit great variation in the quantity of acid, I do not say; there is however, the possibility, that the plant had here been left so long in the dark (48 hours) that the acid produced was itself decomposed. The acid values found are in their relation to the values for p_H somewhat small as compared with the corresponding values for a number of other succulents; for Kleinia $p_H = 4.6$ with corresponding litmus value 24.86 cc. $^n/_5$ per 100 g. of sap, and for Cotyledon linguæfolia $p_H = 4.6$; litmus value = 9.40. This would seem to suggest that Aloe arborescens is not of especially succulent nature, if we thereby understand, for present purposes, the power of accumulating large quantities of acid and exhibiting great variations therein.

ALOE ARBORESCENS Mill.

Table 12.

	g. leaves	% dry matter	% water	pH Lakmoïd-test before	pH Lakmoïd-test after	pH Electrom. measurement Hasselbalch's method	Direct titration values: Litmus cc. $^n/_5$	Direct titration values: Phenolpht. cc. $^n/_5$	Litmus cc. $^n/_5$ per 100 g. sap	Phenolpht. cc. $^n/_5$ per 100 g. sap	Phenolpht. ÷ Litmus	mg. N per 100 g. sap
Left in light; exam. 31 Aug. 1912	189.50	4.29	95.71	5.0	5.0	4.65	1.21	1.74	4.84	6.96	2.12	3.60
48 hours in darkness; examined 2 September	207.75	4.47	95.53	5.0	5.0	4.69	1.23	1.75	4,92	7.00	2.08	3.96

The concentration of hydrogen ions lies within the same field as for the remaining species investigated.

The electrometric measurement by Hasselbalch's method gave here, as in the case of several of the other plants, results showing that the first portion of the liquid exhibited a slightly higher degree of acidity than the succeeding portions, the value here falling from $p_H = 4.59$ to $p_H = 4.65$ [1]), whereafter it remained constant. The cause of this peculiarity I have not been able to discover; in any case, however, we may doubtless suppose that no great quantity of carbonic acid can have been dissolved in the sap, as the results should in such case exhibit a decrease in the numerical values for p_H with the changing of the liquid in the electrode tank.

13. ALOE CYMBÆFOLIA Schrad.

In the case of this plant, there was only sufficient material for a single experiment, the result of which showed the plant to be characterised by a comparatively low acid content, with a concentration of hydrogen ions falling within the same field as found in the case of all the remaining plants.

ALOE CYMBÆFOLIA Schrad.

Table 13.

	g. leaves	% dry matter	% water	pH Lakmoïd-test before	pH Lakmoïd-test after	pH Electrom. measurement Hasselbalch's method	Direct titration values: Litmus cc. n/5	Direct titration values: Phenolpht. cc. n/5	Litmus cc. n/5 per 100 g. sap	Phenolpht. cc. n/5 per 100 g. sap	Phenolpht. ÷ Laknus
1. Left in light; exam. 12 June 1912 3.30	33.00	1.94	98.06	5.2	5.4	5.59	0.24	0.91	1.32	5.02	3.70
2. 23 hours in darkness; exam. 13 June 1912 2.30	52.24	2.24	97.76	4.9	5.0	4.69	0.82	1.51	3.57	6.57	3.00

[1]) Only in the case of Kleinia was the difference thus found greater than that here mentioned.

14. AEONIUM HAWORTHII WEBB & BERTH.

On the 13th August, the temperature reached 20°, falling thence to 13° during the night, and rising again to 30° by noon of the following day.

This experiment, like the two previous ones, is only of value as showing that the concentration of hydrogen ions in this plant moves within the same field as is characteristic for the Crassulaceæ and Aizoaceæ examined. In contrast to the two Liliaceæ just mentioned, Aeonium exhibits a considerable acid content, with a high degree of variation therein. The differences between phenolphtalein and litmus values were very considerable, but there was also in this case a marked precipitation on titration to the last stage.

AEONIUM HAWORTHII WEBB & BERTH.

TABLE 14.

	g. leaves	% dry matter	% water	pH Lakmoid-test: before	pH Lakmoid-test: after	pH Electrom. measurement Hasselbalch's method	Direct titration values: Litmus cc. n/5	Direct titration values: Phenolpht. cc. n/5	Litmus cc. n/5 per 100 g. sap	Phenolpht. cc. n/5 per 100 g. sap	Phenolpht. ÷ Litmus	mg. N per 100 g. sap
a. Left in light; exam. 13 August 1912	54.00	4.11	95.89	4.6	4.7	4.49	6.62	12.40	25.20	47.20	22.00	2.80
b. 23 hours in darkness; exam. 14 August	66.00	3.70	96.30	4.1	4.1	4.13	16.69	23.97	44.21	63.50	19.29	4.20

Summary of Results of the Experiments with Succulents.

a. Some remarks on the substances which determine the difference between titration values for litmus and phenolphtaleïn points.

As we have seen, from Tables 1—14, the titration to the two stages $p_H = 6.81$ and $p_H = 9.2$ showed highly dissimilar values, and the mean difference in these — if we can properly speak of a mean at all where the variations, at any rate in the case of some plants, are so great — varied greatly for the different species of succulents. As to what substances are respon-

sible for the difference, this has as yet only partially been ascertained. First of all, it was necessary to investigate the question as to how the malic acid behaves during titration.

On titrating a solution of malic acid with sodium hydroxide and litmus paper or phenolphtaleïn respectively as indicator, no difference will be found in the two titration values. Thus the values obtained were:

litmus ($p_H = 6.81$)	phenolphtaleïn ($p_H = 9.2$)
11.58 cc. n/10	11.60 cc. n/10.

Malic acid is, however, found not only in its free state in the sap, but also in the form of salts, combined with potassium, sodium, calcium, magnium and aluminium; it will therefore be necessary also to ascertain what takes place with these salts during titration.

The potassium salts will of course exhibit no difference on titration to the two stages in question, since the sodium salts, according to the foregoing, did not do so.

The same applies to the calcium malate.

Twenty cc. of a solution containing 1.623 mg. equival. calcium hydroxide + 1.682 mg. equival. malic acid, showed the following figures:

litmus	phenolphtaleïn
0.59 cc. n/10	0.61 cc. n/10.

The liquid remained clear during and after titration.

Twenty cc. of a solution of magnium malate with a slight surplus of malic acid showed the following titration values:

litmus	phenolphtaleïn
1.47 cc. n/10	1.54 cc. n/10.

The fully titrated liquid remained clear.

Finally, as regards the aluminium malate, a somewhat different result was here obtained:

Twenty cc. of a solution[1]) containing:

0.44099 mg. equivalent aluminium
0.40411 — — potassium
0.85000 — — malic acid

(i. e. with a surplus of 0.0490 mg. equivalent malic acid = 0.49 cc. n/10 malic acid) showed the following titration values:

[1]) The preparation and use of this and the following solution will be subsequently described.

litmus	phenolphtaleïn
2.54 cc. n/10	4.83 cc. n/10.

Of the malic acid combined with aluminium, 2.54 ÷ 0.49 = 2.05 cc. n/10 was titrated to the litmus point, the total quantity combined with aluminium answering to 4.4099 cc. n/10, whence it follows that the 46.49 %, or about half the malic acid combined with the aluminium, was titrated therewith to the mentioned point. Titration to the phenolphtaleïn point was carried out with 4.38 ÷ 0.49 = 3.89 cc. n/10 or 88.21 % of the malic acid combined with aluminium. Similarly, 20 cc. of another solution, containing:

0.6224 mg. equivalent aluminium
0.3797 — — sodium
1.3546 — — malic acid,

showed the following values:

litmus	phenolphtaleïn
7.25 cc. n/10	9.30 cc. n/10,

of which 3.52 cc. n/10 went to titration of the malic acid surplus.

Of the malic acid in combination with aluminium, the following titrations were made:

to litmus..... 3.73 cc. n/10 or 59.97 %
- phenolphtaleïn. 5.78 - - - 92.93 %

Titration to the phenolphtaleïn red will, with a liquid containing aluminium, take some little time, as the red tinge first obtained pales again, and it is not until after repeated droppings of sodium hydroxide that a permanent colour is obtained. This must naturally be explained as due to the fact that the liberation of aluminium hydroxide during titration is not an instantaneous process.

This feature is illustrated by the following: 15 cc. of a solution of malic acid and various malates including, *inter alia*, 4.26 mg. equivalent aluminium malate per 100 cc., was first titrated to the litmus point, and n/10 sodium hydroxide then rapidly added until the desired red appeared. The flask was then well closed with a tightly fitting cork; five minutes later, the bright red colour had given place to a pale pink. More sodium hydroxide was then added until the deep red colour returned; the flask was again closed, and five minutes after, a further addition of sodium hydroxide was found necessary, the colour

having once more faded. The following figures show the course of the titration:

litmus	phenolphtaleïn		
3.02 cc. n/10	5.05 cc. n/10	5.96 cc. n/10	6.10 cc. n/10
	At 1.40	At 1.45	At 1.50

By 1.55, the red had not paled, and the titration was then considered complete. The liquid, which had been clear at first, became gradually clouded by the aluminium hydroxide precipitate.

From these experiments it follows that the presence of aluminium malate will in some degree serve to explain the difference in the titration values in question, and we further see, that this substance is partly responsible for the fact that the titrations to both stages are uncertain. (Some additional experiments, subsequently to be described, will further illustrate this uncertainty in the titration of pure solutions of malic acid and malates.) The titration values obtained for the various succulents, and the figures for aluminium content of the sap likewise show the uncertainty attaching to these titrations — considerably greater, by the way, in the case of the plant sap itself than in the pure malate solutions, as will be seen from the following. Thus for Cotyledon linguæfolia (Table 2 b p. 22) we have in No. 1, 8.40 cc. as the difference between the two titration values per 100 cc. of sap. The same quantity of sap was found to contain 3.03 mg. equivalent aluminium, which is to say, the quantity of malic acid combined with the aluminium answers to $3.03 \times 5 = 15.15$ cc. n/5. The difference between the two titration values corresponds to a good half of this, whereas in the case of the pure malate solutions, we have a titration between litmus and phenolphtaleïn points of between one-half and one-third Experiment No. 2 with Cotyledon linguæfolia gave similar results. If we now take the experiments with Cotyledon obvallata (Table 4 b p. 28) and Rochea falcata (Table 7 b p. 38) from the same point of view, we find in some cases the figures for difference in titration slightly lower than was to be expected, in other cases higher; for Rochea falcata in several instances considerably higher. This seems to indicate that there must, in the case of this last-named plant especially, be other substances present which are likewise titrated to the phenolphtaleïn point. The first to suggest itself is carbonic acid, which will probably always be present in varying quantities

in the sap, since it plays so great a part in the metabolism, both as regards the process of respiration, and that of carbonic acid assimilation. This is, however, of no particular interest, as other substances will here also have to be taken into account, which fact has been demonstrated by various experiments with Rochea falcata. As already mentioned, when titrating the juices to phenolphtaleïn point, there was always a precipitate of more or less dark-coloured matter, this being, in most cases, — and especially with Rochea — very abundant, whereas in the titration of pure malate solutions, only aluminium hydroxide was deposited. That the dark matter thus precipitated is of interest in this connection will be evident from the following:

A portion of Rochea sap, which after centrifuging and filtration was quite clear, and of a light yellow colour, showed under the lakmoïd test $p_H = 4.7$ 12 cc. showed on titration:

litmus	phenolphtaleïn	phenolphtaleïn ÷ litmus
2.41 cc. $^n/_5$	8.66 cc. $^n/_5$	6.25 cc. $^n/_5$

The precipitate obtained on titration was filtered off and washed once or twice in water. The filtrate and the washing water were titrated back to litmus point, consuming 0.46 cc. $^n/_5$ HCl. The substances responsible for the difference in the titration figures must then be in the precipitate.

A portion of sap, 55 cc., was precipitated with 220 cc. abs. alcohol, giving a white deposit of calcium malate and magnium malate. This was filtered off, and 60 cc. of the filtrate, answering to 12 cc. of the original sap, showed on titration:

litmus	phenolphtaleïn	phenolphtaleïn ÷ litmus
2.25 cc. $^n/_5$	9.51 cc. $^n/_5$	7.26 cc. $^n/_5$

The difference for phenolphtaleïn ÷ litmus was slightly increased. To the remainder of the alcoholic filtrate, 30 cc. $^n/_5$ sodium hydroxide was then added, giving an abundant deposit. The filtrate from this, which gave an alkaline reaction when applied to litmus paper, but not to phenolphtaleïn, gave no further precipitate when treated with sodium hydroxide. The precipitate was washed a little, mixed up with water, and then dissolved in $^n/_5$ hydrochloric acid. This gave a clear, claret-coloured liquid, which shortly after, however, again became clouded, and the deposit now formed proved insoluble on the addition of a further quantity of hydrochloric acid. The fact that the colour of the

liquid during these processes; precipitation with sodium hydroxide and solution of the deposit in hydrochloric acid, had changed from pale yellow to claret colour suggests that some chemical changes, possibly oxidation, had taken place.

In order to ascertain whether oxidation had occurred, the following experiment was made:

15 cc. of the clear yellow Rochea sap showed the following titration values:

	litmus	phenolphtaleïn	phen.÷ litm.
	2.47 cc. $^{n}/_{5}$	7.69 cc. $^{n}/_{5}$	5.22 cc. $^{n}/_{5}$
per 100 cc. sap:	16.47 - -	51.27 - -	34.80 - -

The titrated liquid was now left for an hour in atmospheric air, after which, 7.69 cc. $^{n}/_{5}$ hydrochloric acid was added, this quantity answering to the $^{n}/_{5}$ sodium hydroxide used in titration to last stage. The precipitate, which had become somewhat dark in the meantime, did not dissolve. Titration was resumed, with the following results:

	litmus	phenolphtaleïn	phen.÷ litm.
	3.20 cc. $^{n}/_{5}$	7.85 cc. $^{n}/_{5}$	4.65 cc. $^{n}/_{5}$
per 100 cc. sap:	21.33 - -	52.33 - -	31.00 - -

Two portions of 22 cc. of the same sap were placed, one in a current of hydrogen, and one in a current of atmospheric air, and 11.28 cc. $^{n}/_{5}$ sodium hydroxide added, this being the quantity calculated from the above titration values as required to bring the liquid to the phenolphtaleïn point. In both portions, an abundant precipitate was formed, that in the portion exposed to ordinary atmosphere becoming rapidly dark, while that in the hydrogen current remained light.

One hour later, 11.28 cc. $^{n}/_{5}$ hydrochloric acid was added to each portion, corresponding to the amount of sodium hydroxide originally added, and titration was carried out as follows:

		litmus	phenolpht.	phen.÷litm.
In hydrogen		4.21 cc. $^{n}/_{5}$	9.95 cc. $^{n}/_{5}$	5.74 cc. $^{n}/_{5}$
	per 100 cc. sap.	19.14 - -	45.23 - -	26.09 - -
In ordinary atmosphere		5.15 - -	10.40 - -	5.25 - -
	per 100 cc. sap.	23.41 - -	47.27 - -	23.86 - -

Titration to the litmus point was throughout fairly sharp, but that to phenolphtaleïn somewhat uncertain, owing to the

difference in colour of the precipitate in the separate portions. The experiment showed, that the litmus value was distinctly increased in both portions, undoubtedly more so in that exposed to ordinary atmosphere. The phenolphtaleïn values had somewhat decreased. The results appear to indicate that formation of strong acids takes place, this being, however, to a lesser degree the case with the hydrogen portion than with the remainder.

Similar experiments gave the following results per 100 cc. sap:

	litmus	phenolpht.	phen ÷ litm.
Titrated at once	20.00 cc. n/5	37.90 cc. n/5	37.90 cc. n/5
$2^1/_2$ hours in atmospheric air free from carbonic acid	32.50 - -	69.00 - -	36.50 - -
$2^1/_2$ hours in hydrogen current	18.79 - -	53.79 - -	35.00 - -

The hydrogen portion was then filtered; the filtrate and the precipitate mixed with water were separately oxidised for 48 hours in a current of air free from carbonic acid, whereupon titration of the two liquids showed:

	phen.÷ litmus
Filtrate	2.64 cc. n/5
Precipitate	20.93 - -

This experiment likewise showed an increase in the strong acids upon oxidation, whereas in the hydrogen current, no increase took place. We also find, that the substances of interest in this connection are precipitated at a concentration of hydrogen ions of less than $10^{\div 7}$.

Finally, experiments were made with a view to ascertaining what influence is exerted upon the substances in question by dilution of the sap.

	litmus	phenolphtalein
15 cc. sap	2.40 cc. n/5	8.31 cc. n/5
15 cc. sap + 35 cc. H_2O	2.52 - -	6.48 - -

Corresponding portions were then titrated in a hydrogen current, left 48 hours in a current of air free from carbonic acid, and again titrated, after addition of the quantity of sodium hydroxide answering to that used in titration.

15 cc. sap:	litmus	phenolpht.	phen.÷litm.
Titrated at once in hydrogen current	2.54 cc. n/5	8.31 cc. n/5	5.77 cc. n/5
48 hours in atmosph. free from carbonic acid	7.29 - -	9.78 - -	2.49 - -
15 cc. sap + 135 cc. water: Titrated at once in hydrogen current	2.55 - -	6.47 - -	3.92 - -
48 hours in atmosph. free from carbonic acid	6.35 - -	8.72 - -	2.37 - -

An entirely similar experiment showed the following results:

15 cc. sap:	litmus	phenolpht.	phen.÷litm.
Titrated at once in hydrogen current	1.97 cc. n/5	11.35 cc. n/5	9.38 cc. n/5
72 hours in atmosph. free from carbonic acid	8.15 - -	14 88 - -	6.73 - -
15 cc. sap + 135 cc. H_2O: Titrated at once in hydrogen current	1.94 - -	8.13 -	6.19 - -
72 hours in atmosph. free from carbonic acid	6.94 - -	12.82 - -	5.88 - -

These experiments show that a tenfold dilution of the sap occasions no essential alteration in the litmus figures, but effects a reduction in the quantity of those substances acting as weak acids, or at least in such of these as can be titrated to the phenolphtaleïn point.

From these few experiments, then, we find that we have here to deal with compounds of a highly inconstant nature. It is therefore not surprising that the titration to phenolphtaleïn point proved, in the case of several plants, a matter of considerable difficulty, and frequently gave only uncertain results, or that the red colour characteristic of phenolphtaleïn ions in alkaline solution is in various ways and to varying degree liable to be obscured. It would seem likely that they are of considerable importance in the metabolism of the plants, since they occur in such quantities; further investigation on this point would also be of great interest. In a subsequent chapter, some additional experiments with these substances will be described.

It will be seen from Tables 1—14, that the quantity of nitrogenous compounds occurring in solution in the cell-sap of the plants examined stands in no relation whatever either to the concentration of hydrogen ions or to the titration values. As to whether they are or are not partly responsible for the difference between the two titration values, I do not venture to say, but this is by no means impossible, as it is characteristic of polypeptides that they show a marked difference between litmus and phenolphtalein points, appearing as acids with a concentration of hydrogen ions of less than 10^{-7}[1]). This is believed to be due to certain intramolecular rearrangements at low concentrations of hydrogen ions. For glycylglycin

CH_2NH_2 we have probably the following rearrangement: CH_2NH_2
|
CO
|
NH
|
CH_2
|
COOH

|
COH
||
N
|
CH_2
|
COOH

and similar rearrangements are supposed to take place with the remaining polypeptides, as the linking of peptide, which in acid substrate occurs with the ketone form — CO — NH —, should on alkaline reaction pass to the enol form — C(OH) = N —, having the character of a weak acid (S. P. L. Sørensen)[2]).

If we now assume, as an extreme case, that for each atom of N, a group of acid character appears, then this would occasion a difference between the two titration values of $^5/_{14}$ cc. $^n/_5$ = 0.36 cc. $^n/_5$ for each mg. of N. For Diotostemon Hookeri, we found, in No. 2, a total nitrogen content of 4.45 mg., which would thus account for a difference of $4.45 \cdot 0.36 = 1.60$ cc. $^n/_5$ between the phenolphtaleïn and litmus figures; on titration, however, a far more marked divergence was noted, to wit, 19.60 cc. $^n/_5$. Similar results were found in the case of the remaining Crassulaceæ and in Aeonium Haworthii; in the Mesembrianthemum species, on the other hand, a highly abundant content of soluble nitrogenous compounds was found, with a very slight difference

[1]) S. P. L. Sörensen and Henriques, Zeitschr. f. physiol. Chemie **63**, 27—40 (1909).

[2]) Ergebnisse der Physiologie **12**, 478 (1912).

between the two titration values; we have thus here to consider the possibility that the mentioned difference is due to the nitrogenous compounds. In some few cases, formol titrations were carried out[1]); in Mesembrianthemum lingaeforme for instance, 1 a and b showed respectively 5.44 and 2.72 mg. of nitrogen which could be formol titrated, whereas in Mesembrianthemum echinatum Exp. 2, no measurable quantity of nitrogen amenable to formol titration could be discerned. True, the weak acids had previously been precipitated with barium hydroxide after addition of barium chloride, which of course might have rendered it impossible afterwards to measure groups that could otherwise have been titrated with formol; the experiments suggest, however, that we cannot consider the polypeptides as an essential cause of the difference between the titration values.

A single quantitative determination of phosphorus was made with sap of Rochea falcata, according to the Neumann method, as modified by Gregersen[2]). 100 g. of sap was found to contain a quantity answering to 3.38 cc. $^{mol}/_{15}$ H_3PO_4. Even if we reckon with the total quantity of phosphorus as present in the form of acid, only about half this quantity will be titrated between the litmus and phenolphtaleïn points, a part having been titrated before the litmus point is reached. The phosphorus will thus in any case be of no essential importance as regards the difference in titration values.

From all this, then, it appears that in the first place, aluminium malate occasions a certain difference between the two titration values employed, this difference being possibly also in a lesser degree due to dissolved nitrogenous compounds, to carbonic acid, and phosphoric acid. Finally however, the highly inconstant substances which were specially investigated in the case of Rochea falcata are of considerable importance in this connection. It is these substances in particular which render titration of plant juices to the phenolphtaleïn point uncertain or valueless.

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[1]) These were, however, soon discontinued, owing to the slight nitrogen content in the sap of most of the plants treated.

[2]) Zeits. f. physiol. Chemie **53**. 453 (1907).

b. The concentration of hydrogen ions dependent upon the quantity of acid which can be titrated to the litmus point.

From the comparative chart (Fig. 11) in which all litmus curves for the various plants are shown together, it will be seen that the curves for low concentration of hydrogen ions all lie near the abscissa, whereas those for higher concentration of hydrogen ions rise more or less steeply upward In other words, the quantities of acid found in the various species at low concentration of hydrogen ions do not differ greatly one from another, but exhibit a continually increasing divergence as the concentration of hydrogen ions rises. By following the ordinate line corresponding to abscissa 4.00, we find the following values for quantity of acid in the various species:

Crassula lactea.....	45 cc. $^{n}/_{5}$	per 100 g. sap
Crassula obovata.....	46 - -	—
Rochea falcata.....	55 - -	—
Kleinia.....	60 - -	—

and similarly, for abscissa 4.50:

Mesembrianthemum lingaeforme.....	6 cc. $^{n}/_{5}$
Cotyledon obvallata.....	12 - -
Cotyledon linguæfolia.....	12 - -
Diotostemon Hookeri.....	15 - -
Mesembrianthemum echinatum.....	18 - -
Crassula lactea.....	23 - -
Rochea falcata.....	24 - -
Cotyledon coruscans.....	25 - -
Crassula obovata.....	25 - -
Kleinia.....	30 - -

The plants also arrange themselves in very much the same order with regard to quantity of acid at a p_H value of 5.0, save that the differences between the values are here far less significant, the quantity of acid varying between 2 and 15. And finally, for abscissa 5.5, the quantity of acid varies only between abt. 1 and abt. 5 cc. $^{n}/_{5}$. From this it follows, that the power of producing and accumulating acid differs to a very high degree in different plants; albeit this power does not, as stated by Aubert, stand in any absolute relation to the degree of fleshi-

ness in the various species. Kleinia, which stands first among the plants here investigated with regard to acid production, has probably the least fleshy leaves af all; Cotyledon obvallata, Cotyledon linguæfolia and Mesembrianthemum lingaeforme have all particularly thick and juicy leaves, but are nevertheless among the lowest in respect of acid secretion.

The fact that the various species differ greatly in this respect will be clearly apparent if we glance at some experiments carried out with different plants, but simultaneously, and under like conditions, as for instance Exp. 5 and 7 p. 49 with Mesembrianthemum lingaeforme, and Exp. 3 and 4 p. 25 with Cotyledon coruscans. In the case of the first-named plant, the quantity of acid increased during the experimental period of 23 hours (for the last 20 hours of which the plant has been left in darkness) from 3.23 to 5.95 cc. $^n/_5$; in Cotyledon coruscans, on the other hand, from 34.37 to 46.02 cc. $^n/_5$. The increase was thus 2.72 and 11.65 cc. $^n/_5$ respectively. Together with this, the two plants showed an alteration in the concentration of hydrogen ions of from $10^{\div 4.83}$ to $10^{\div 4.61}$ and from $10^{\div 4.30}$ to $10^{\div 4.07}$ respectively.

As already mentioned, all the litmus curves are characterised by a more or less uniform appearance, all falling more or less steeply towards the abscissa at low concentrations of hydrogen ions. This feature, which is very distinctly apparent in Fig. 11, being common to all the plants investigated, the question naturally arises as to what should be the cause. In taking up this question for investigation, it should first of all be noted that the p_H values, as already mentioned, are closely related to the quantities of acid which can be titrated to litmus point, which will for the most part be equivalent to the quantity of malic acid, this being the predominant acid in succulents, as several investigators have shown. In addition to names previously quoted, Aberson[1]) and E. Schmidt[2]) have demonstrated that malic

[1]) Aberson showed that the malic acid in Crassulaceæ answers to the formula:

COOH
|
CHOH
|
CH_2
|
COOH;

i. e. having the C-atoms in a normal carbon series. (Ber. d. d. chem. Ges. **31**. **2**. 1432 (1898)).

[2]) Archiv der Pharmacie, **224**. 535 (1886).

acid is predominant among the Crassulaceæ, whereas Berg and Gerber[1]) point out that in various Mesembrianthemum species (e. g. Mes. lingaeforme) the greater part of the acid found is malic acid, and not oxalic, as generally believed.

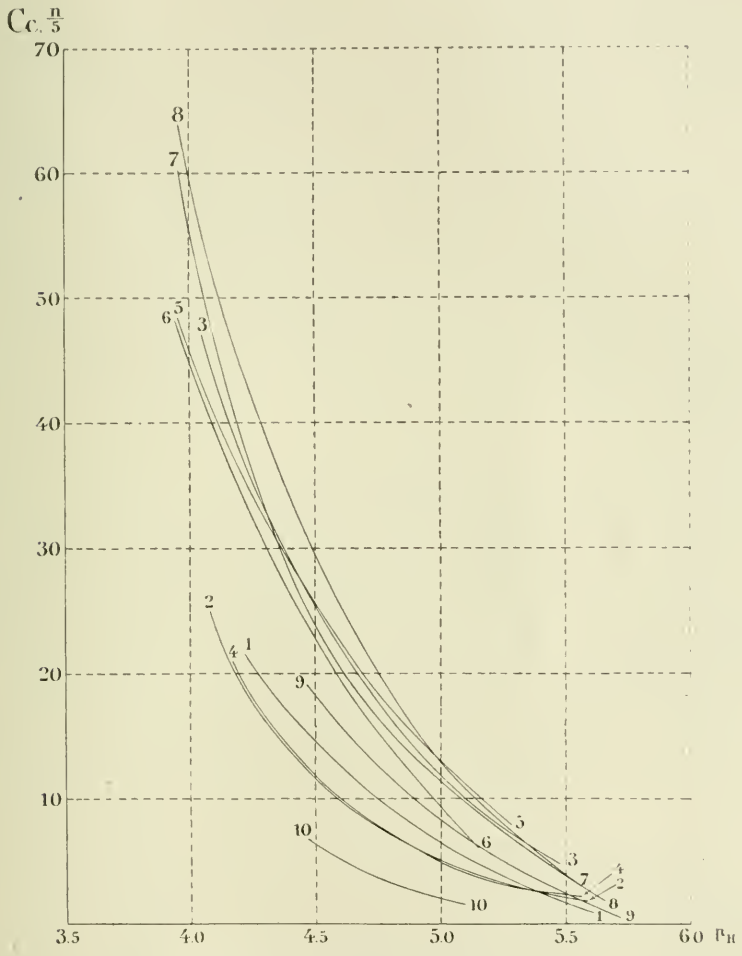

Fig. 11. Comparative chart.

It must also be borne in mind that these plants contain, not only acids, but also salts of such acids. We have thus, in the sap, to reckon with the occurrence of normal salt, acid salt and free acid, and the p_H values are therefore dependent not

[1]) Revue Générale de Botanique, **8**. 295 (1896).

only upon the quantity of acid which can be titrated, but also on the quantity of salts.

Determination of the bases found in the sap showed that the quantity of these is fairly constant within one and the same species, but differs greatly from one species to another. Thus in the three species investigated, it was found that Rochea is far richer in bases than the two Cotyledon species, which latter are more or less alike in this respect. These differences in the quantity of bases however, correspond in a remarkable manner to the degree in which the three species in question secrete and accumulate acid, and to the quantity of acid required in the different species to bring about the same concentration of hydrogen ions. Further explanation of this interesting feature, which affords no inconsiderable aid to the comprehension of what factors determine the extent of acid serection, will be given in the following chapter, as it will be necessary first to give some theoretical observations, and describe certain experiments illustrative of the same.

c. Concentration of hydrogen ions dependent upon the relation between quantity of acid and quantity of salts.

In various recent works, notably those of S. P. L. Sörensen[1]) and L. J. Henderson[2]), frequent mention is made of the fact that a mixture of a weak acid with its salt produced an effect, as of a "buffer", the addition of even a strong acid or base in reasonable quantity making no essential alteration in the concentration of hydrogen ions where such a "buffer" mixture is present. The magnitude of the concentration of hydrogen ions found in the solution, and determined by the buffer solution, will depend partly upon the dissociation constant of the acid in question, and partly upon the relation between the quantities of acid and of its salt.

Now the sap of the succulents here investigated contains, as we have seen from the foregoing, a mixture of malic acid and one or more of its salts, which must act as a buffer; the concentration of hydrogen ions in the sap must therefore be de-

[1]) Compt. rend. d. Laborat. d. Carlsberg **8**. 35 (1909); Ergebnisse der Physiologie. **12**. 407, 424 ff. (1912).

[2]) Ergebnisse der Physiologie. **8** 254—325 (1909).

pendent partly upon the dissociation constants of the malic acid, and partly upon the relation between the quantity of malic acid present and the quantity of malates.

The amount of basic substances present in the ash can presumably be taken as an approximate measure of the quantity of salts contained in the sap of the plant in question; the relation here concerned may therefore also be expressed as that between the number of milligramme equivalents of titratable acid present in a given quantity of sap, say 100 g. or 100 cc., and the number of milligramme equivalents of base in the amount of ash corresponding to a like quantity of sap. The relation in question may in other words be briefly formulated as equal to "the number of equivalents of titratable acid per equivalent of base". This proportion will in the following be denoted as O.

In the foregoing section, where it was desired to compare the results obtained by measurement of the concentration of hydrogen ions with those from titration, the value of p_H was used in the graphs as abscissa, and the titration value, answering to the acid content of the sap, as ordinate. In the following, it will be natural, after what we have already observed, to take the value of p_H as abscissa and O as ordinate.

The entire question here to be investigated will be far more simple and clear in the case of a monobasic acid and its salt of a monovalent base than if we have to take, as in the case of the plant juices, the dibasic malic acid and its salts with bases of different valence. I have therefore thought it best to begin by considering a series of simple mixtures, and have selected for this purpose the well-known buffer mixtures of acetic acid and sodium acetate.

a. Monobasic acid and its salt of a monovalent base.

For mixtures of acetic acid with its sodium salt, we can according to the law of mass action write the following equation:

$$[H]^+ \cdot [Ac]^- = k \cdot [HAc],$$

where k denotes the dissociation constant of the acid.

The hydrogen ions are here derived solely from the acid, whereas the acetate ions are derived both from the acid and from the salt. As long as we have to deal with a weak acid and its sodium salt, and the quantity of salt present is not inconsiderable in proportion to the amount of acid, then the por-

tion of acetate ions derived from acid will be slight in comparison with that from the salt, as sodium salts are always highly dissociated, and the formula can therefore be written approximately thus:

$$b = k \frac{[\text{Acetic acid}]}{[\text{Sodium acetate}]} = k \cdot \frac{A}{\alpha \cdot B} \text{ (I)},$$

b indicating the concentration of hydrogen ions, A that of the titratable acid, B, the concentration of salt, and α the degree of dissociation of the same.

If the dissociation is complete, i. e. $\alpha = 1$, then b will be proportional with the relation between concentration of acetic acid and concentration of sodium acetate, i. e. with O. The curve expressing the dependence between concentration of hydrogen ions and O will then be a straight line, whereas that for the dependence between values of p_H and O assumes a logarithmic form. Calculations of such curves will be found in Table 15; the curves themselves in Figs. 12 a and 12 b.

Table 15.

Calculations of hydrogen ion concentration in solutions of sodium acetate and acetic acid according to formula $b = k \frac{A}{\alpha \cdot B}$; $k = 1.8 \cdot 10 \div 5$.

No.	$\frac{A}{B} = O$	$\alpha = 1$		$\alpha = 0.80$		$\alpha = 0.60$	
		b	pH	b	pH	b	pH
1	0.05	$0.09 \cdot 10 \div 5$	6.05	$0.11 \cdot 10 \div 5$	5.96	$0.15 \cdot 10 \div 5$	5.82
2	0.10	0.18	5.75	0.22	5.66	0.30	5.52
3	0.20	0.36	5.44	0.45	5.35	0.60	5.22
4	0.30	0.54	5.27	0.68	5.17	0.90	5.05
5	0.40	0.72	5.14	0.90	5.05	1.20	4.92
6	0.50	0.90	5.05	1.12	4.95	1.50	4.82
7	0.60	1.08	4.97	1.35	4.87	1.80	4.74
8	0.80	1.44	4.84	1.80	4.74	2.40	4.62
9	1.00	1.80	4.74	2.25	4.65	3.00	4.52
10	1.20	2.16	4.67	2.70	4.57	3.60	4.44
11	1.60	2.88	4.54	3.60	4.44	4.80	8.32
12	2.00	3.60	4.44	4.50	4.35	6.00	4.22
13	2.40	4.32	4.36	5.40	4.27	7.20	4.14
14	2.80	5.04	4.30	6.30	4.20	8.40	4.08

Where α is less than 1, and supposing that its value depends entirely upon the concentration of sodium acetate, not also upon the concentration of acetic acid, which we may probably assume, without great error, to be the case, then we obtain, as expression of the dependence between b or p_H respectively and O, a group of straight lines in the one case and a group of logarithmic curves in the other, each corresponding to its sodium acetate concentration with the appropriate value of α. Calculations for such groups of curves will be found in Table 15, the curves being drawn from α values of 0.80 and 0.60. The curves themselves are shown in Figs. 12 a and 12 b.

As already mentioned, the formula here employed is only approximately correct, the quantity of acetate ions derived from the titratable acid being disregarded. The complete formula may, however, be arrived at in the following manner:

From the law of mass action, we have as above:

$$[H] \cdot [Ac] = k\ [HAc].$$

If now b denote, as before, the concentration of hydrogen ions, then b will express not only this, but also the concentration of the acetate ions derived from the titratable acid, since a dissociation of the acid gives an equal number of hydrogen ions and acetate ions. The acetate ions derived from the salt may as before be denoted by $\alpha \cdot B$, B being the total salt concentration, and α the degree of dissociation of the salt at the concentration in question. The total concentration of acetate ions will then be $[b + \alpha B]$. As a measure of a non-dissociated acid, we have $A \div b$, this being the difference between the total quantity of acid A and that dissociated, which of course is measured by b. Inserting these values in the formula, we obtain:

$$b\,(b + \alpha B) = k\ (A \div b)$$

whence again:

$$b = \div {}^1/_2\,(\alpha B + k) + {}^1/_2 \sqrt{(\alpha B + k)^2 + 4\,k\,A} \quad (II).$$

This formula has been used for calculating the concentration of hydrogen ions in three series of mixtures, containing 10 cc., 1 cc. and 0.1 cc. mol. sodium acetate per 100 cc. of solution, and with varying quantities of acetic acid. The values have been calculated partly with an α magnitude of 1.0 and partly with a magnitude of 0.80 for the same. From the table (Table 16) it

will be seen that the values coincide with those found by the approximate formula (I) as long as B is 0.1, and the number of figures reckoned with is not greater than here; if, on the other hand, B be 0.1 or 0.001, there will be a difference in the results,

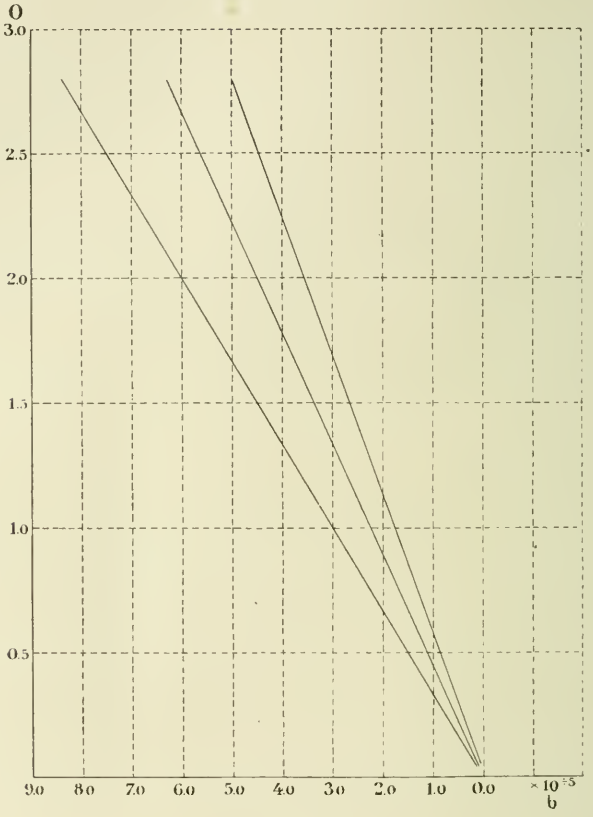

Fig. 12a.

save in the first cases, where A is very low. The fact that the approximate formula can be used to such an extent as here is of course due to the slight value of the dissociation constant; in the case of stronger acids, i. e. acids with higher dissociation constants, the practical utility of the reduced formula would be more restricted.

Our consideration of the simplest case (that of a monobasic acid with a monovalent base), which was primarily intended to serve as an explanatory introduction to the following, is now concluded, and we can pass on to deal with the more com-

plicated instances, i. e. those of dibasic acids with their salts, having monovalent or bivalent bases, these being the ones of practical utility in the investigation of what takes place in the sap.

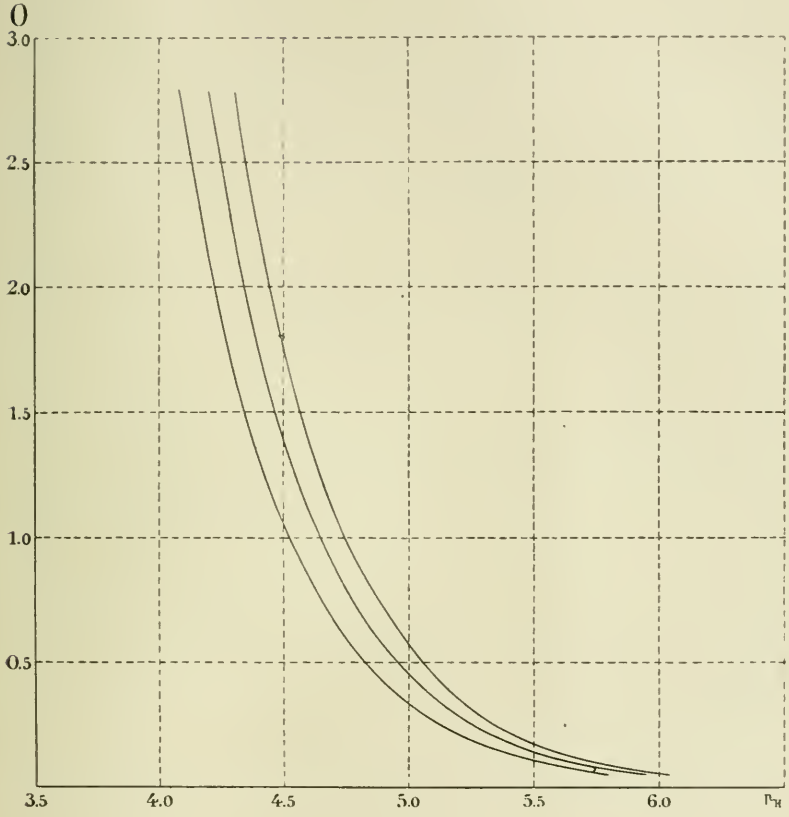

Fig. 12 b.

β. Dibasic acid and its salt of a monovalent base.

In the case of a dibasic acid, the question becames more complicated than when dealing with a monobasic; the concentration of hydrogen ions will here be dependent, not upon one only, but upon two dissociation constants, in addition to which, the solution may here contain, besides the free acid, both normal and acid salt as well, and, where the acid is combined with bivalent or polyvalent bases, possibly also several kinds of acid salt.

We may take first of all the case of an acid combined with

TABLE 16.

Calculation of concentration of hydrogen ions in solutions of sodium acetate and acetic acid, according to the formula:

$$b = \div \tfrac{1}{2}(\alpha B + k) \pm \tfrac{1}{2}\sqrt{(\alpha B + k)^2 + 4kA};\quad k = 1.8 \cdot 10 \div 5.$$

100 cc. contains		Molal. concentration		Titration acidity expressed in mg. equivalents per 100 cc. sol.	O = Titration acidity per mg. equivalent base in 100 cc.	Calculated concentration of hydrogen ions			
						$\alpha = 1$		$\alpha = 0.80$	
mol. acetic acid cc.	mol. sodium acetate cc.	A	B			b	pH	b	pH
1.00	10.00	0.010	0.100	1.00	0.10	$0.18 \cdot 10 \div 5$	5.75	$0.22 \cdot 10 \div 5$	5.66
5.00	10.00	0.050	0.100	5.00	0.50	$0.90 \cdot 10 \div 5$	5.05	$1.12 \cdot 10 \div 5$	4.95
10.00	10.00	0.100	0.100	10.00	1.00	$1.80 \cdot 10 \div 5$	4.74	$2.25 \cdot 10 \div 5$	4.65
20.00	10.00	0.200	0.100	20.00	2.00	$3.60 \cdot 10 \div 5$	4.44	$4.50 \cdot 10 \div 5$	4.35
0.10	1.00	0.0010	0.010	0.10	0.10	$0.18 \cdot 10 \div 5$	5.75	$0.22 \cdot 10 \div 5$	5.65
0.50	1.00	0.0050	0.010	0.50	0.50	$0.90 \cdot 10 \div 5$	5.05	$1.12 \cdot 10 \div 5$	4.95
1.00	1.00	0.0100	0.010	1.00	1.00	$1.79 \cdot 10 \div 5$	4.75	$2.24 \cdot 10 \div 5$	4.65
2.00	1.00	0.0200	0.010	2.00	2.00	$3.58 \cdot 10 \div 5$	4.45	$4.47 \cdot 10 \div 5$	4.35
0.01	0.10	0.0001	0.001	0.01	0.10	$0.18 \cdot 10 \div 5$	5.75	$0.22 \cdot 10 \div 5$	5.66
0.05	0.10	0.0005	0.001	0.05	0.50	$0.88 \cdot 10 \div 5$	5.06	$1.09 \cdot 10 \div 5$	4.96
0.10	0.10	0.0010	0.001	0.10	1.00	$1.74 \cdot 10 \div 5$	4.76	$2.14 \cdot 10 \div 5$	4.67
0.20	0.10	0.0020	0.001	0.20	2.00	$3.42 \cdot 10 \div 5$	4.47	$4.19 \cdot 10 \div 5$	4.38

a monovalent base. Distinction must here be made between 1) solutions containing normal salt together with acid salt, and 2) those containing acid salt together with free acid.

1) If, in a solution consisting of acid salt MHS and normal salt M_2S, we call the concentration, reckoned in mol., of the acid salt B, the concentration of the normal salt C, the degree of dissociation of the metallic atom of the acid salt α, and that for the two metallic atoms of the normal salt α' and β respectively, the concentration of hydrogen ions b, then, since the ion $(HS)^{\div}$ thrown off from the acid salt may be dissociated in $H^+ + S^{\div\div}$, according to the law of mass action, we have,

$$[H]^+ \cdot [S]^{\div\div} = k_2 [HS]^{\div}$$

where k_2 denotes the second dissociation constant of the acid.

Here, of course, the hydrogen ions can only be derived from the acid salt, as also the $(HS)^{\div}$ ions, whereas the $S^{\div\div}$ ions may originate as well from the acid salt as from the normal salt. The quantity of $S^{\div\div}$ ions derived from the acid salt may be taken as $= b$, the $(HS)^{\div}$ ion giving an equal quantity of hydrogen ions and $S^{\div\div}$ ions, and we may with a high degree of probability assume that the dissociation of the acid salt

$$(MHS) \longrightarrow (MS)^{\div} + (H)^+$$

is altogether insignificant.

The quantity of $S^{\div\div}$ ions derived from the normal salt can be measured by $\alpha \cdot \beta \cdot C$ and the total concentration of $S^{\div\div}$ ions will then be $b + \alpha' \beta C$. The quantity of $(HS)^{\div}$ ions, which will of course be derived solely from the acid salt, must be $\alpha B \div b$, this indicating the difference between that part of the acid salt which has only thrown off its metallic atom, and that portion which has also thrown off its hydrogen atom.

Inserting these values in the foregoing equation, we obtain:

$$b \cdot (b + \alpha' \beta \cdot C) = k_2 (\alpha B \div b) \quad \text{(III)}.$$

As long as we have not to deal with strong acids, and neither B nor C approach too nearly to 0, we may take b as infinitesimal compared with B and C, and the equation will then appear as follows:

$$b = k_2 \frac{\alpha \cdot B}{\alpha' \cdot \beta \cdot C},$$

from which we may probably cancel out α as against α', at any rate where B and C do not differ too greatly one from the other. The equation will then be:

$$b = k_2 \frac{B}{\beta \cdot C} \quad \text{(IV)}.$$

Without these restrictions, the equation (III) appears thus:

$$b = \div {}^1/_2 (\alpha' \beta C + k_2) \div {}^1/_2 \sqrt{(\alpha' \beta C + k_2)^2 + 4 k_2 \alpha B} \quad \text{(V)}.$$

2) In solutions containing acid salt MHS together with free acid H_2S, the following dissociations may take place:

for the acid: $H_2S \rightarrow H^+ + (HS)^\div$
and $(HS)^\div \rightarrow H^+ + S^{\div\div}$
For the acid salt: $MHS \rightarrow M^+ + (HS)^\div$
and $(HS)^\div \rightarrow H^+ + S^{\div\div}$.

We may probably here, as before, omit the dissociation:

$$MHS \rightarrow H^+ + (MS)^\div.$$

By the law of mass action we obtain:

$$[H]^+ [HS]^\div = k_1 [H_2S]$$
$$\text{and } [H]^+ [S]^{\div\div} = k_2 [HS]^\div$$

where k_1 and k_2 denote the first and second dissociation constants of the acid respectively.

Taking b as usual to denote the concentration of hydrogen ions, and indicating the concentration of the acid salt reckoned in mol. by B, that of the acid by 1, A the degree of dissociation of the acid salt by α, and the concentration of $S^{\div\div}$, by q, we obtain, by insertion in the foregoing equations

$$b (\alpha B + b \div 2q) = k_1 (A \div (b \div q)) \quad \text{(VI)}.$$
$$\text{and } b \cdot q = k_2 (\alpha B + b \div 2q) \quad \text{(VII)}.$$

It will easily be seen, that the concentration of $(HS)^\div$ must be $(\alpha B + b \div 2q)$, since this ion is derived partly from the acid salt (B) and partly from the acid, which gives an equal quantity of hydrogen ions and $(HS)^\div$ ions, of which latter q is further dissociated into hydrogen ions and $S^{\div\div}$ ions.

From equations VI and VII, we can eliminate q, and then find b:

$$\frac{b (\alpha B + b) \div k_1 (A \div b)}{k_1 + 2b} = \frac{k_2 (\alpha B + b)}{2k_2 + b} \quad \text{(VIII)},$$

whence again we obtain:

$$b^3 + b^2 (\alpha B + k_1) + b (k_1 k_2 \div k_1 A) \div k_1 k_2 (2A + \alpha B) = 0 \quad \text{(IX)}.$$

In solutions where neither A nor B approach too nearly to

0, we may take b as infinitesimal compared with A and B, and equation (VIII) will then be simplified as follows:

$$\frac{b \cdot \alpha B \div k_1 A}{k_1 + 2b} = \frac{k_2 \alpha B}{2k_2 + b'}$$

whence again we obtain:

$$b = \frac{k_1 A \pm \sqrt{k_1^2 A^2 + 4 k_1 k_2 \alpha B (2A + \alpha B)}}{2 \alpha B} \quad (X).$$

These equations may now be utilised for the calculation of concentration of hydrogen ions in a series of mixtures of a dibasic acid with its salts; the quantity of base is maintained constant within this series, the quantity of acid varying in the different portions. The calculations are first made for tartaric acid, the two dissociation constants of this being known, as:

$$k_1 = 9.70 \cdot 10^{\div 4} \text{ and } k_2 = 5.9 \cdot 10^{\div 5}\text{[1]}.$$

In these calculations, we first presuppose complete dissociation of the salts; i. e. $\alpha = \alpha' = \beta = 1$. The results are shown in Table 17. For the calculation of Nos. 1—6, the approximate formula (IV), has been used, the complete formula (V) having been found to give exactly the same results. Nos. 7—10 were obtained partly by the complete formula (V) and partly by the approximate (IV); the results obtained by means of the last-named are placed in parantheses, and it will be seen, that only in the case of No. 10 does the result obtained by the complete formula differ from that given by the approximate one to such a degree as to preclude the employment of the latter. A difference of 0.02 in p_H is practically insignificant, as the measurements which can be made by the methods hitherto employed are subject to a limit of error amounting to just c. 0.02 in p_H.

For Nos. 11—19, formula (X) has been employed, a test in the case of No. 11 having shown that the complete formula (IX) gave the same result as (X).

The results obtained are drawn up in the co-ordinate system shown in Fig. 13. The course of the corresponding curves I a and I b appear to indicate, however, that the formulæ employed are no longer valid when we approach the purely acid salts, i. e. for solutions of acid salt and acid, where the quantity of acid

[1]) W. A. Smith: Zeits. f. physik. Chemie. **25**. 234 (1898).

TABLE 17.

Calculation of concentration of hydrogen ions in solutions of tartaric acid with its salts of a monovalent base. Reckoning with values $k_1 = 9.7 \cdot 10 \div 4$ and $k_2 = 5.9 \cdot 10 \div 5$. In calculation, formula (IV) used for Nos. 1—6, (V) for Nos. 7—10 and (X) for Nos. 11—19. All solutions contain 10 mg. equivalent base. See Curves I a and b Fig. 13.

No.	100 cc. solution contains			Molal concentration			Titration acidity expressed in mg equivalents per 100 cc. solution	O	Calculated concentration of hydrogen ions $\alpha = \beta = 1$	
	mol. tartaric acid cc.	mol. MH tartrate cc.	mol. M_2 tartrate cc.	A	B	C			b	pH
1	—	0.50	4.75	—	0.0050	0.0475	0.50	0.05	$6.2 \cdot 10 \div 6$	5.21
2	—	1.00	4.50	—	0.0100	0.0450	1.00	0 10	$1.3 \cdot 10 \div 5$	4.88
3	—	2.00	4.00	—	0.0200	0.0400	2.00	0.20	$2.9 \cdot 10 \div 5$	4.53
4	—	3.00	3.50	—	0.0300	0.0350	3.00	0.30	$5.1 \cdot 10 \div 5$	4.30
5	—	4.00	3.00	—	0.0400	0.0300	4.00	0.40	$7.9 \cdot 10 \div 5$	4.10
6	—	5.00	2.50	—	0.0500	0.0250	5.00	0.50	$1.2 \cdot 10 \div 4$	3.93
7	—	6.00	2.00	—	0.0600	0.0200	6.00	0.60	$1.7 \cdot 10 \div 4$	3.76 (3.75)[1]
8	—	7.00	1.50	—	0.0700	0.0150	7.00	0.70	$2.7 \cdot 10 \div 4$	3.57 (3.56)[1]
9	—	8.00	1.00	—	0.0800	0.0100	8.00	0.80	$4.5 \cdot 10 \div 4$	3.35 (3.33)[1]
10	—	9.00	0.50	—	0.0900	0.0050	9.00	0.90	$8.9 \cdot 10 \div 4$	3.05 (2.97)[1]
11	1.00	10.00	—	0.0100	0.1000	—	12.00	1.20	$3.15 \cdot 10 \div 4$	3.50 { (b — $3.12 \cdot 10 \div 4$; pH = 3.51)[2]
12	1.25	10.00	—	0.0125	0.1000	—	12.50	1.25	$3.3 \cdot 10 \div 4$	3.48
13	1.50	10.00	—	0.0150	0.1000	—	13.00	1.30	$3.5 \cdot 10 \div 4$	3.45
14	2.00	10.00	—	0.0200	0.1000	—	14.00	1.40	$4.0 \cdot 10 \div 4$	3.40
15	2.50	10.00	—	0.0250	0.1000	—	15.00	1.50	$4.4 \cdot 10 \div 4$	3.36
16	3.00	10.00	—	0.0300	0.1000	—	16.00	1.60	$4.8 \cdot 10 \div 4$	3.32
17	5.00	10.00	—	0.0500	0.1000	—	20.00	2.00	$6.6 \cdot 10 \div 4$	3.18
18	7.00	10.00	—	0.0700	0.1000	—	24.00	2.40	$8.3 \cdot 10 \div 4$	3.08
19	9.00	10.00	—	0.0900	0.1000	—	28.00	2.80	$1.0 \cdot 10 \div 3$	2.99

[1]) Figures in parentheses obtained by means of formula (IV).

[2]) Figures in parentheses obtained by means of formula (XI).

is insignificant in comparison with the acid salt, and in solutions of acid salt with normal salt, where the latter is present in very slight quantities compared with the former. The values for 9

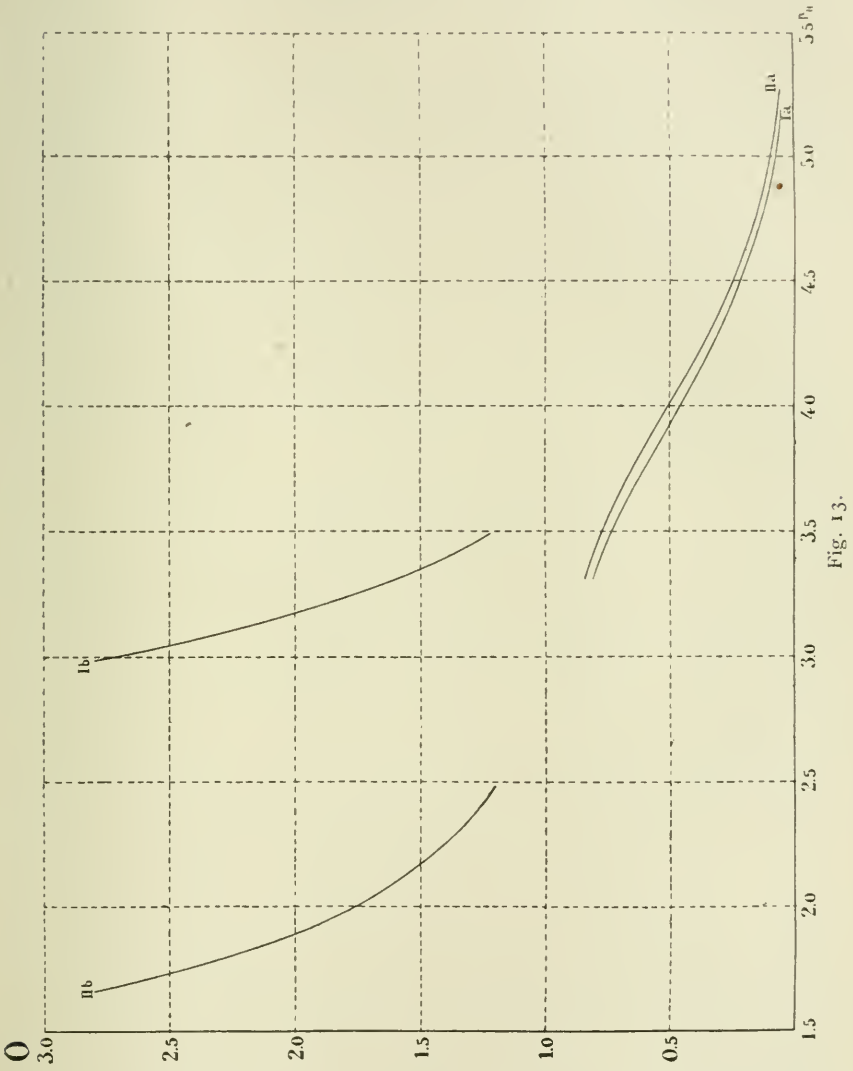

Fig. 13.

and 10, corresponding to solutions of normal salt together with acid salt, show a higher degree of acid than those for 11—15, the value for 10 being even more acid than those for 16—18, despite the fact that all mixtures answering to the same values contained far more acid at the same quantity of base. The cause

of this I am unable to determine, but curves found by empirical methods when compared with calculated curves for malic acid and its salts, which will be referred to later on, confirm the above indication that the formulæ cannot apply in the cases mentioned.

Up to the present, we have presupposed complete dissociation of the salts, and on this supposition, it will be seen from the formulæ (IV) and (X), which may, as we have seen, be used instead of the complete formulæ, at any rate where the dissociation constants of the acid are not essentially higher than that of tartaric acid, that b is independent of the absolute values of A, B and C, when these do not approach O, and is determined solely by the ratio between these magnitudes. In formula (IV) we can thus write r instead of $\frac{B}{C}$ and then obtain: $b = k_2 \frac{r}{\beta} = k_2 \cdot r$, when $\beta = 1$. Writing then, in formula (X) p instead of $\frac{A}{B}$, we obtain:

$$b = \frac{k_1 p \pm \sqrt{k_1^2 p^2 + 4 k_1 k_2 \alpha (2p + \alpha)}}{2\alpha}$$

or, for $\alpha = 1$ $b = \frac{k_1 p \pm \sqrt{k_1^2 p^2 + 4 k_1 k_2 (2p + 1)}}{2}$

As O may be expressed by

$$\frac{B}{B + 2C} = \frac{B:C}{B:C + 2} = \frac{r}{r + 2}, \text{ or by } \frac{2A + B}{B} = 2p + 1,$$

according as we have to deal with solutions of acid salt and normal salt or solutions with acid salt and acid, it will be seen that O is likewise determined solely by the ratio between A and B, or B and C respectively, and not by the absolute values of these. Hence, however, it follows, that as long as O is kept constant, b will also be constant, i. e. independent of the absolute concentrations, provided the salts are completely dissociated; in such case, the dependence of the hydrogen ion concentration upon O may be expressed by a single curve. It is directly apparent from the formulæ, that when the dissociation is not complete, this dependent relation will not be represented by a single curve, but, as we saw in the case of the acetic acid, by a group of curves, each curve answering to its own particular concentration of the base, which again determines the degree of dissociation. In the case of malic acid, which will shortly be

TABLE 13.

Calculation of concentration of hydrogen ions in solutions of oxalic acid with its salts of a monovalent base. Reckoning with values $k_1 = 3.8 \cdot 10 \div 2$ og $k_2 = 4.9 \cdot 10 \div 5$. In calculation, formula (IV) used for Nos. 1—6, (V) for Nos. 7—10 and (IX) for Nos. 11—18. All solutions contain 10 mg. equivalent base See Curves IIa and b Fig. 13.

No.	100 cc. solution contains			Molal concentration			Titration acidity expressed in mg. equivalents per 100 cc. solution	O	Calculated concentration of hydrogen ions $\alpha = \beta = 1$	
	mol. oxalic acid cc.	mol. MH oxalate cc.	mol. M_2 oxalate cc.	A	B	C			b	pH
1	—	0.50	4.75	—	0.0050	0.0475	0.50	0.05	$5.2 \cdot 10 \div 6$	5.29
2	—	1.00	4.50	—	0.0100	0.0450	1.00	0.10	$1.1 \cdot 10 \div 5$	4.96
3	—	2.00	4.00	—	0.0200	0.0400	2.00	0.20	$2.5 \cdot 10 \div 5$	4.61
4	—	3.00	3.50	—	0.0300	0.0350	3.00	0.30	$4.2 \cdot 10 \div 5$	4.38
5	—	4.00	3.00	—	0.0400	0.0300	4.00	0.40	$6.5 \cdot 10 \div 5$	4.19
6	—	5.00	2.50	—	0.0500	0.0250	5.00	0.50	$9.8 \cdot 10 \div 5$	4.01
7	—	6.00	2.00	—	0.0600	0.0200	6.00	0.60	$1.46 \cdot 10 \div 4$	3.84 (3.83) [1]
8	—	7.00	1.50	—	0.0700	0.0150	7.00	0.70	$2.25 \cdot 10 \div 4$	3.65 (3.64) [1]
9	—	8.00	1.00	—	0.0800	0.0100	8.00	0.80	$3.76 \cdot 10 \div 4$	3.43 (3.41) [1]
10	—	9.00	0.50	—	0.0900	0.0050	9.00	0.90	$7.59 \cdot 10 \div 4$	3.12 (3.06) [1]
11	1.00	10.00	—	0.0100	0.1000	—	12.00	1.20	$3.2 \cdot 10 \div 3$	2.50 (2.36) [2]
12	1.50	10.00	—	0.0150	0.1000	—	13.00	1.30	$4.4 \cdot 10 \div 3$	2.36 (2.22) [2]
13	2.00	10.00	—	0.0200	0.1000	—	14.00	1.40	$5.6 \cdot 10 \div 3$	2.25 (2.10) [2]
14	2.50	10.00	—	0.0250	0.1000	—	15.00	1.50	$6.8 \cdot 10 \div 3$	2.17 (2.01) [2]
15	3.00	10.00	—	0.0300	0.1000	—	16.00	1.60	$8.0 \cdot 10 \div 3$	2.10 (1.93) [2]
16	5.00	10.00	—	0.0500	0.1000	—	20.00	2.00	$1.3 \cdot 10 \div 2$	1.89 (1.69) [2]
17	7.00	10.00	—	0.0700	0.1000	—	24.00	2.40	$1.7 \cdot 10 \div 2$	1.77 (1.57) [2]
18	9.00	10.00	—	0.0900	0.1000	—	28.00	2.80	$2.2 \cdot 10 \div 2$	1.66 (1.46) [2]

[1]) Figures in parentheses obtained by means of formula (IV).

[2]) Figures in parentheses obtained by means of formula (X).

referred to, such groups of curves have been drawn, these being partly empirically found, partly obtained by theoretical calculation.

In the case of oxalic acid also, calculations have been made as to the concentration of hydrogen ions in mixtures with like quantities of base, both with increasing quantity of acid. The results are shown in Table 18, and the corresponding curves in Fig. 13, II a and II b. The second dissociation constant for oxalic acid $4.9 \cdot 10^{\div 5}$ is slightly lower than the second constant for tartaric acid $5.9\ 10^{\div 5}$, whereas the first, $380 \cdot 10^{\div 4}$ on the other hand, is far higher than the corresponding value for tartaric acid, $(= 9.7 \cdot 10^{\div 4})$. In agreement with this, we find that the curve calculated for oxalic acid, answering to mixtures of acid salt together with normal salt, very nearly coincides with the corresponding calculated curve for tartaric acid, the first showing a slightly more alkaline direction. In the case of the curves for acid salt and free acid, on the other hand, we find a very considerable difference between the two acids, the oxalic lying far more to the acid side than the tartaric. Finally, the first dissociation constant for oxalic acid is so high that the calculation of the curve for acid salt with free acid cannot be made according to the reduced formula (X) but requires the complete formula (IX), which will be seen from the table.

Malic acid and its salts.

We may now pass on to consider malic acid and its salts, this acid being here of interest as constituting the principal part of the acids present in the sap of the succulents investigated. The first dissociation constant of malic acid has been ascertained by Ostwald by means of determinations of conductivity as $3.95 \cdot 10^{\div 4}$ at a temperature of 25^0 for the levo acid[1]). For the inactive form of the acid, Ostwald[2]), and later W. A. Smith[3]) found the value of $3.99 \cdot 10^{\div 4}$ for k_1.

A regards the second dissociation constant, W. A. Smith found the value $k_2 = 8.3 \cdot 10^{\div 6}$[4]), which he arrived at by means of Noyes' formula:

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[1]) Zeits. f. physikal. Chemie. **3**. 370 (1889).
[2]) *l. c.* p. 371.
[3]) Zeits. f. physikal. Chemie. **25**. 193 (1898).
[4]) *l. c.* p. 234.

$$(k_2) = c = \frac{(k + m + H)\,H^2}{k\,(m + H)} \text{ [1]},$$

where m denotes the concentration of the acid salt, k the first dissociation constant of the acid, and H the concentration of hydrogen ions[2]. The magnitude k was here determined by measurements of conductivity as $3.99 \cdot 10^{-4}$, while H was arrived at by the inversion method, based on the fact that the velocity of inversion of a solution of cane sugar with sufficiently diluted acid solutions is proportional to the concentration of hydrogen ions. The solution employed had a concentration of 1 gramme equivalent in 128 l., and the concentration of hydrogen ions found was $0.56 \cdot 10^{-4}$. We cannot, however, take it for granted that the value found for $k_2 = c$ is certain, in the first place because the inversion method is not altogether reliable, especially in the case of highly diluted solutions, and in the second place, because Noyes' formula presupposes a complete dissociation of the sodium ions of the acid salt.

That the determination of k_2 by this means is not a little uncertain would also appear from the fact that Smith found, for the second dissociation constant of tartaric acid, a value of $59 \cdot 10^{-6}$, whereas McCoy[3]) obtained the widely different value of $34 \cdot 10^{-6}$. McCoy also employed Noyes' formula, but did not measure the concentration of hydrogen ions by the inversion

[1]) Noyes arrived at this formula by the following process:

In a solution of an acid salt of a dibasic acid, the following elements may occur: MHA, M+, (HA)−, H+, A−−, H_2A and M_2A. In highly diluted solutions, the concentrations of MHA and M_2A are insignificant, and may therefore be disregarded. The following equations may be formed: $c\,[HA] = [H]\,[A]$ (1) and $k \cdot [H_2A] = [H]\,[HA]$ (2). As the negative ion A is derived from the following two reactions: $2\,HA = H_2A + A$ and $HA = H + A$, we obtain: $[A] = [H] + [H_2A]$ (3); $m = [HA] + [H_2A] + [A]$ (4), where m indicates the concentration of the salt; (the quantities of MHA and M_4A are disregarded). From (1) and from (3) and (4) it follows, that:

$$[HA] = \frac{[H][A]}{c} = m + [H] - [2A];\quad [H]\,[A] = cm + c\,[H] - 2c\,[A] \text{ (5)}.$$

From (1) and from (2) and (3) we obtain:

$$[HA] = \frac{[H][A]}{c} = \frac{k\,[A] - k\,[H]}{[H]};\quad [A] = \frac{ck\,[H]}{ck - [H]^2} \text{ (6)};$$

inserting this value in (5), we have then:

$$ck = \frac{(k + m + [H])\,[H]^2}{m - [H]}$$

[2]) Zeits. f. physikal. Chemie. **11**. 495 (1893).

[3]) Journ. Am. Chem. Soc. **30** 688 (1908).

method, calculating it instead from the electrical conductivity of the solution. We do not yet know which value is the more correct.

The second dissociation constant of malic acid has further been determined by Datta and Dhar[1]) as $0.903 \cdot 10^{-5}$, according to a method based on the fact that a solution of the normal sodium salt of a dibasic acid can absorb more carbonic acid than the same volume of water, as the natrium salt of the dibasic acid becomes combined with carbonic acid, and from this difference in the quantity of carbonic acid, the dissociation constant of the acid may be arrived at. I have myself carried out a determination of k_2 by measurements of the concentration of hydrogen ions in mixtures of normal and acid sodium malate.

For these mixtures, a normal solution of sodium hydroxide, free from carbonic acid, was used, this being prepared in the following manner: Sodium hydroxide *e natrio* from Kahlbaum was dissolved in water free from carbonic acid to a solution of abt. 17 n; this was decanted off from the precipitated sodium carbonate, diluted in a flask fllled with air free from carbonic acid, and the strength determined by sodium oxalate[2]).

As regards the malic acid, a preparation from Kahlbaum was used. An analysis of this showed, that 2 g. of substance contained 0.0017 g. of ash, i. e. 0.085 % ash. The malic acid was then investigated for possible anhydride, two like solutions being titrated, one without boiling, the other after being boiled with a slight surplus of sodium hydroxide solution. The quantity consumed was:

	unboiled	boiled
I.	31.09 cc. n/5 NaOH	31.10 cc. n/5 NaOH
II.	48.75 cc. n/5 NaOH	48.73 cc. n/5 NaOH

i. e. no anhydride was found.

Finally, the malic acid was investigated for other calcium precipitating acids (oxalic, tartaric and citric acid) according to the method before described. Neither oxalic nor citric acid could be discerned; there was, however, perhaps a very slight trace of tartaric acid.

From this malic acid preparation, the solutions were made

[1]) Journ. Chem. Soc. London. **107**. 824 (1915).
[2]) Medd. fra Carlsberg Lab. **8**. 33 (1909).

up with distilled water free from carbonic acid, kept in a copper receptacle closed with a tube containing soda-lime.

On mixing the malic acid solution with the mentioned solution of sodium hydroxide in calculated quantities, a solution of sodium malate was obtained, and from this again, by addition of calculated quantities of malic acid and water, the solutions used for measuring the concentration of hydrogen ions.

A solution of calcium malate, likewise used for determination of concentration of hydrogen ions, the results of which will be shortly referred to, was prepared as follows: From calcium hydroxide, washed in water, a saturated calcium hydroxide solution was made up, this being filtered down into a 2 l. volumetric flask free from carbonic acid until the flask was filled to the mark. 50 cc. of the solution was titrated, and a quantity of calcium hydroxide as thereby calculated poured out into an equivalent amount of malic acid, giving a solution of calcium malate with known concentration. This was evaporated down to $^{mol}/_{40} = {}^{n}/_{20}$. And from this the mixtures used for measurement were prepared (as a rule, 100 cc. of each mixture). In cases where only 2 or less than 2 cc. of the acid was to be added to the calcium malate or sodium malate solutions, the acid was first highly diluted, and the equivalent quantity then added.

In the calculations, the equation IV shown on p. 93 $b = k_2 \frac{B}{\beta \cdot C}$, was used, this giving, as we have seen, the relation between concentration of hydrogen ions in a liquid with known quantities of normal and acid salt of a dibasic acid and the second dissociation constant of the acid in question. Of the magnitudes in this equation, B and C are known; b can be measured, and we have thus remaining k_2, which must be calculated, and β. The value for β, i. e. the degree of dissociation of the normal salt on dissociation of the second metallic atom, is, it is true, unknown, but we shall hardly make any great error in supposing that it does not differ greatly from the degrees of dissociation in the sodium salts of monovalent organic acids in corresponding dilution, as it has been found that these were very nearly alike one to the other. These values may be calculated from the equivalent conductivity of the salts in question; the relation between the conductivity at a given degree of dilution μ_v and infinite dilution $\mu\infty$, i. e. that at which the dissociation

is complete, indicates the degree of dissociation. Determinations of degree of conductivity μ_v for the sodium salts of various organic acids have *inter alia* been made by Bredig, and some of these may here be quoted, the calculated values which I myself have obtained for the degrees of association $= \frac{\mu_v}{\mu\infty}$ being noted at the same time.

v=	Sodium acetate		Sodium lactate		Sodium glycolate[1])	
No. of litres per Gramme molecule	μ_v	$\frac{\mu_v}{\mu\infty}$	μ_v	$\frac{\mu_v}{\mu\infty}$	μ_v	$\frac{\mu_v}{\mu\infty}$
32	75.5	0.86	69.6	0.85	74 0	0.85
64	77.6	0.89	71.6	0.87	76.4	0.88
128	79 8	0.91	74.1	0.90	79.0	0.91
256	81.6	0.93	76.4	0.93	81.2	0.94
512	83.5	0.95	78.2	0.95	83.0	0.96
1024	85.0	0.97	79.8	0.97	84.7	0.98
∞	87.5	—	82.1	—	86.8	—

(Bredig, Zeits. f. physik. Ch. **13**. 218 (1894).

This table shows, that the sodium salts of the three organic acids mentioned have throughout practically the same degree of dissociation. Now the values given above were obtained at a temperature of 25°, but the following table of measurements for sodium acetate at 18° shows very nearly corresponding degrees of dissociation.

Gramme equivalents per litre	μ_v	$\frac{\mu_v}{\mu\infty}$
0.0005	(75.8)	0.97
0.001	(75.2)	0.96
0.002	74.3	0.95
0 005	(72.4)	0.92
0.01	70.2	0.89
0.02	67.9	0.87
0.03	(66.3)	0.85
0.05	64.2	0.82
0.1	61.1	0.78

(Landolt und Börnstein, p. 1103.)

[1]) That Bredig's measurements, and not the more "classic" ones given by Ostwald, are here quoted, is due to the fact that Bredig states the value of $\mu\infty$.

No value for $\mu\infty$ is here given, but as we know the migratory velocities for the sodium ion and the acetate ion at 18°, $\mu\infty$ at 18° may easily be calculated, the conductivity of a substance being a purely additive quality, determined by the sum of the conductivity of the ions; for these migratory velocities, we have λ na = 43.5, λ acetate = 35 (Kohlrausch and Steinwehr) whence $\mu\infty$ = 78.5 [1]).

These degrees of dissociation may doubtless be applied, without risk of serious error, to sodium malate, albeit this may not be quite correct. There is every reason to suppose that the sodium salts of dibasic acids will have a somewhat inferior degree of dissociation, since Ostwald, Walden and others have found, that the conductivity increases at a higher rate on dilution with the sodium salts of dibasic acids than with those of the monobasic. Thus, we find, in the sodium salts of monobasic acids, a difference of c. 10 in the values for μ on dilution to 32 and 1024 l. respectively per g. equivalent salt, while for the sodium salts of dibasic acids, the difference is abt. $2 \cdot 10$ and $4 \cdot 10$ respectively for salts of dibasic acids with monovalent base or monobasic acids with divalent base and for salts of dibasic acids with divalent base.

In solutions $^{mol}/_{32} = {}^{n}/_{16}$, β has been reckoned either at 0.80, interpolated from the foregoing table, or 0.75, and in solutions of twice this degree of dilution, a value either of 0.85 or 0.80. True, the solutions employed were only $^{n}/_{16}$ and $^{n}/_{32}$ respectively as regards the malate ion, while the base varied from $^{n}/_{18}$ to $^{n}/_{21}$ in the first 4 cases, and was $^{n}/_{30}$ in the fifth, but the magnitude of possible error here may doubtless be regarded as infinitesimal, which would also appear from the following.

The values obtained by measurement for concentration of hydrogen ions are shown in Table 19, which also gives the calculated values for k_2 from the formula $k_2 = \frac{b \cdot \beta C}{B}$.

The mean figures for these five values are $1.19 \cdot 10^{\div 5}$ and $1.12 \cdot 10^{\div 5}$. There is thus no great difference in k_2, whichever degree of dissociation we reckon with.

Measurements of concentration of hydrogen ions were also made in solutions of the pure acid salt, and the values used for calculation of k_2 according to Noyes' formula (cf. p. 81) k_1 being reckoned as $= 4.00 \cdot 10^{\div 4}$.

TABLE 19.

No.	Composition of solution			Molal concentration		Concentration of hydrogen ions as found		$k_2 = \frac{b \cdot \beta \cdot C}{B}$		O = Titration acidity mg. equiv. base
	$mol/_{32}$ NaH malate cc.	$mol/_{32}$ Na_2 malate cc.	H_2O cc.	of acid salt B	of normal salt C	b	pH	for $\beta = 0.80$	for $\beta = 0.75$	
1	10	30	—	0.007813	0.023438	$5.13 \cdot 10 \div 6$	5.29	$1.23 \cdot 10 \div 5$	$1.15 \cdot 10 \div 5$	0.14
2	15	25	—	0.011719	0.019530	$8.91 \cdot 10 \div 6$	5.05	$1.19 \cdot 10 \div 5$	$1.12 \cdot 10 \div 5$	0.23
3	20	20	—	0.015625	0.015625	$1.41 \cdot 10 \div 5$	4.85	$1.13 \cdot 10 \div 5$	$1.06 \cdot 10 \div 5$	0.33
4	20	30	—	0.012500	0.018750	$1.02 \cdot 10 \div 5$	4.99	$1.22 \cdot 10 \div 5$	$1.15 \cdot 10 \div 5$	0.25
								for $\beta = 0.85$	for $\beta = 0.80$	
5	5	15	20	0.003906	0.011719	$4.57 \cdot 10 \div 6$	5.34	$1.17 \cdot 10 \div 5$	$1.10 \cdot 10 \div 5$	0.14

The values found were:

			p_H	k_2	
mol/128	NaH	malate	4.19	$1.11 \cdot 10^{\div 5}$	
mol/256	—	—	4.25	$0.90 \cdot 10^{\div 5}$	Mean value
mol/512	—	—	4.30	$0.79 \cdot 10^{\div 5}$	$k_2 = 0.93 \cdot 10^{\div 5}$

It will be seen that the values thus found for k_2 do not agree very well, appearing to decrease with increasing dilution. It is then a question whether greater uniformity can be expected. And here it should, in the first place, be borne in mind, that Noyes' formula presupposes complete dissociation of the sodium atoms, which, however, we cannot be sure is here the case, when we take into consideration the figures for conductivity given by Bredig and Kohlrausch (p. 84). And in the second place, it must be remembered, that the degree of accuracy with which concentration of hydrogen ions can be measured is such that the values can vary by 1 millivolt; it will therefore be necessary to ascertain what influence such variation can have upon the result for k_2.

In the last named case, the value found for k_2 was $0.79 \cdot 10^{\div 5}$ when p_H was 4.30 answering to an electromotive force of $\pi = 0.5856$ volts. If we now give π the value 0.5846, thus decreasing by 1 millivolt, then $p_H = 4.28$ and k_2 will be calculated as $0.87 \cdot 10^{\div 5}$.

Which of the two mentioned features is mainly resporsible for the inaccuracy of the results I do not venture to say, but it would seem likely that it is the former, since this appears to occasion a systematic variation.

In the following, a value of $1.15 \cdot 10^{\div 5}$ will be taken for k_2, in agreement with the result found by the first method, and a value of $4.00 \cdot 10^{\div 4}$ for k_1. This latter, or rather, a value of $3.99 \cdot 10^{\div 4}$ applies, it is true, to a temperature of 25^0, but it is hardly likely that any serious error is incurred by taking the same for 18^0, at any rate, as far as regards our present purpose.

With these values for the two dissociation constants of malic acid, similar calculations have been made as to the concentration of hydrogen ions in mixtures of malic acid with its salts, in the same manner as previously in the case of tartaric and oxalic acid (cf. Table 20); here, however. the calculations have been carried out, not only in the supposition that the dissociation of the kations is complete, but also with given values for degree

TABLE 20.

Calculation of concentration of hydrogen ions in solutions of malic acid with its salts. Reckoned with values: $k_1 = 4.0 \cdot 10 \div 4$; $k_2 = 1.15 \cdot 10 \div 5$. Formula (IV) employed for Nos. 1—6, formulæ (IV) and (V) for Nos. 7—10, formula (X) for Nos. 11—19. Cf. Fig. 14.

No.	100 cc. solution contained			Molal concentration			Titration acidity expressed in mg. equivalents per 100 cc. solution	O	Calculated concentration of hydrogen ions	
									$\alpha = \beta = 1$	
	mol. malic acid cc.	mol. MH malate cc.	mol. M_2 malate cc.	A	B	C			b	pH
1	—	0.50	4.75	—	0.0050	0.0475	0.50	0.05	$1.2 \cdot 10 \div 6$	5.92
2	—	1.00	4.50	—	0.0100	0.0450	1.00	0.10	$2.6 \cdot 10 \div 6$	5.59
3	—	2.00	4.00	—	0.0200	0.0400	2.00	0.20	$5.8 \cdot 10 \div 6$	5.24
4	—	3.00	3.50	—	0.0300	0.0350	3.00	0.30	$9.9 \cdot 10 \div 6$	5.01
5	—	4.00	3.00	—	0.0400	0.0300	4.00	0.40	$1.5 \cdot 10 \div 5$	4.82
6	—	5.00	2.50	—	0.0500	0.0250	5.00	0.50	$2.3 \cdot 10 \div 5$	4.64
7	—	6.00	2.00	—	0.0600	0.0200	6.00	0.60	$3.5 \cdot 10 \div 5$ ($3.5 \cdot 10 \div 5$) [1]	4.46 (4.46) [1]
8	—	7.00	1.50	—	0.0700	0.0150	7.00	0.70	$5.3 \cdot 10 \div 5$ ($5.4 \cdot 10 \div 5$) [1]	4.27 (4.27) [1]
9	—	8.00	1.00	—	0.0800	0.0100	8.00	0.80	$9.0 \cdot 10 \div 5$ ($9.2 \cdot 10 \div 5$) [1]	4.05 (4.04) [1]
10	—	9.00	0.50	—	0.0900	0.0050	9.00	0.90	$1.9 \cdot 10 \div 4$ ($2.1 \cdot 10 \div 4$) [1]	3.72 (3.68) [1]
11	1.00	10.00	—	0.0100	0.1000	—	12.00	1.20	$9.7 \cdot 10 \div 5$	4.01
12	1.25	10.00	—	0.0125	0.1000	—	12.50	1.25	$1.05 \cdot 10 \div 4$	3.98
13	1.50	10.00	—	0.0150	0.1000	—	13.00	1.30	$1.13 \cdot 10 \div 4$	3.95
14	2.00	10.00	—	0.0200	0.1000	—	14.00	1.40	$1.30 \cdot 10 \div 4$	3.89
15	2.50	10.00	—	0.0250	0.1000	—	15.00	1.50	$1.47 \cdot 10 \div 4$	3.83
16	3.00	10.00	—	0.0300	0.1000	—	16.00	1.60	$1.65 \cdot 10 \div 4$	3.78
17	5.00	10.00	—	0.0500	0.1000	—	20.00	2.00	$2.39 \cdot 10 \div 4$	3.62
18	7.00	10.00	—	0.0700	0.1000	—	24.00	2.40	$3.15 \cdot 10 \div 4$	3.50
19	9.00	10.00	—	0.0900	0.1000	—	28.00	2.80	$3.93 \cdot 10 \div 4$	3.41

[1]) Figures in parentheses obtained by means of formula (IV).

TABLE 21.

Calculation of concentration of hydrogen ions in solutions of malic acid with its salts. Reckoned with values: $k_1 = 4.0 \cdot 10^{-4}$; $k_2 = 1.15 \cdot 10^{-5}$. Formula (IV) employed for Nos. 1—9, formula (V) for No. 10, formula (X) for Nos. 11—19. Cf. Fig. 14.

No.	Relative values for			O	Calculated concentration of hydrogen ions							
					$\alpha = \beta = 1$		$\alpha = \beta = 0.80$		$\alpha = \beta = 0.60$		$\alpha = \beta = 0.40$	
	A	B	C		b	pH	b	pH	b	pH	b	pH
1	—	0.50	4.75	0.05	$1.2 \cdot 10^{-6}$	5.92	$1.5 \cdot 10^{-6}$	5.82	$2.0 \cdot 10^{-6}$	5.70	$3.0 \cdot 10^{-6}$	5.52
2	—	1.00	4.50	0.10	$2.6 \cdot 10^{-6}$	5.59	$3.2 \cdot 10^{-6}$	5.50	$4.3 \cdot 10^{-6}$	5.37	$6.4 \cdot 10^{-6}$	5.19
3	—	2.00	4.00	0.20	$5.8 \cdot 10^{-6}$	5.24	$7.2 \cdot 10^{-6}$	5.14	$9.6 \cdot 10^{-6}$	5.02	$1.4 \cdot 10^{-5}$	4.84
4	—	7.00	3.50	0.30	$9.9 \cdot 10^{-6}$	5.01	$1.2 \cdot 10^{-5}$	4.91	$1.6 \cdot 10^{-5}$	4.78	$2.5 \cdot 10^{-5}$	4.61
5	—	4.00	3.00	0.40	$1.5 \cdot 10^{-5}$	4.82	$1.9 \cdot 10^{-5}$	4.72	$2.6 \cdot 10^{-5}$	4.59	$3.8 \cdot 10^{-5}$	4.42
6	—	5.00	2.50	0.50	$2.3 \cdot 10^{-5}$	4.64	$2.9 \cdot 10^{-5}$	4 54	$3.8 \cdot 10^{-5}$	4.42	$5.8 \cdot 10^{-5}$	4.24
7	—	6.00	2.00	0.60	$3.5 \cdot 10^{-5}$	4.46	$4.3 \cdot 10^{-5}$	4.37	$5.8 \cdot 10^{-5}$	4.24	$8.6 \cdot 10^{-5}$	4.06
8	—	7.00	1.50	0.70	$5.3 \cdot 10^{-5}$	4.27	$6.7 \cdot 10^{-5}$	4.17	$8.9 \cdot 10^{-5}$	4.05	$1.3 \cdot 10^{-4}$	3.87
9	—	8.00	1.00	0.80	$9.0 \cdot 10^{-5}$	4.05	$1.15 \cdot 10^{-4}$	3.94	$1.5 \cdot 10^{-4}$	3.81	$2.3 \cdot 10^{-4}$	3.64
10	—	9.00	0.50	0.90	$1.9 \cdot 10^{-4}$	3.72	—	—	—	—	—	—
11	1.00	10.00	—	1.20	$9.7 \cdot 10^{-5}$	4.01	$1.05 \cdot 10^{-4}$	3.98	$1.19 \cdot 10^{-4}$	3.93	$1.47 \cdot 10^{-4}$	3.83
12	1.25	10.00	—	1.25	$1.05 \cdot 10^{-4}$	3.98	—	—	—	—	—	—
13	1.50	10.00	—	1.30	$1.1 \cdot 10^{-4}$	3.95	$1.25 \cdot 10^{-4}$	3.90	$1.47 \cdot 10^{-4}$	3.83	$1.92 \cdot 10^{-4}$	3.72
14	2.00	10.00	—	1.40	$1.3 \cdot 10^{-4}$	3.89	$1.47 \cdot 10^{-4}$	3.83	$1.77 \cdot 10^{-4}$	3.75	$2.39 \cdot 10^{-4}$	3.62
15	2.50	10.00	—	1.50	$1.5 \cdot 10^{-4}$	3.83	$1.69 \cdot 10^{-4}$	3.77	$2.07 \cdot 10^{-4}$	3.68	$2.86 \cdot 10^{-4}$	3.54
16	3.00	10 00	—	1.60	$1.65 \cdot 10^{-4}$	3.78	$1.92 \cdot 10^{-4}$	3.72	$2.39 \cdot 10^{-4}$	3.62	$3.34 \cdot 10^{-4}$	3.48
17	5.00	10.00	—	2.00	$2.4 \cdot 10^{-4}$	3.62	$2.87 \cdot 10^{-4}$	3.54	$3.67 \cdot 10^{-4}$	3.44	$5.30 \cdot 10^{-4}$	3.28
18	7.00	10.00	—	2.40	$3.15 \cdot 10^{-4}$	3.50	$3.83 \cdot 10^{-4}$	3.42	$4.98 \cdot 10^{-4}$	3.30	$7.28 \cdot 10^{-4}$	3.14
19	9.00	10.00	—	2.80	$3.9 \cdot 10^{-4}$	3.41	$4.81 \cdot 10^{-4}$	3.32	$6.29 \cdot 10^{-4}$	3.20	$9.24 \cdot 10^{-4}$	3.03

TABLE 22 a.

Empiric values for concentration of hydrogen ions in mixtures of sodium malate and malic acid. All solutions are $^{mol}/_{40} = {}^{n}/_{40}$ as regards sodium content; 100 cc. contains 2.5 mg. equiv. sodium. Cf. curves I a and b, Fig. 15.

No.	100 cc. solution contained			Molal concentration			Titration figures in cc. $^{n}/_{5}$ per 100 cc. solution	O = mg. equiv. titratable acid : mg. equiv. base	pH
	mol. malic acid cc.	mol. NaH malate cc.	mol. Na_2 malate cc.	A acid	B acid salt	C normal salt			
1	—	0.125	1.1875	—	0.00125	0.011875	0.625	0.05	5.81
2	—	0.250	1.1250	—	0.0025	0.01125	1.250	0.10	5.52
3	—	0.500	1.0000	—	0.0050	0.0100	2.500	0.20	5.18
4	—	0.750	0.8750	—	0.0075	0.00875	3.750	0·30	4.95
5	—	1.000	0.7500	—	0.0010	0.0075	5.000	0.40	4.79
6	—	1.250	0.6250	—	0.0125	0.00625	6.250	0.50	4.64
7	—	1.500	0.5000	—	0.0150	0.0050	7.500	0.60	4.50
8	—	2.000	0.2500	—	0.0200	0.0025	10.000	0.80	4.29
9	—	2.500	—	—	0.0250	—	12.500	1.00	4 13
10	0.25	2.500	—	0.0025	0.0250	—	15.000	1.20	3.97
11	0.75	2.500	—	0.0075	0.0250	—	20.000	1.60	3.75
12	1.25	2.500	—	0.0125	0.0250	—	25.000	2.00	3.59
13	1.75	2.500	—	0.0175	0.0250	—	30.000	2.40	3.48
14	2.25	2.500	—	0.0225	0.0250	—	35.000	2.80	3.38

of dissociation, to wit, 0.8, 0.6 and 0.4 (Table 21). Both dissociation constants for malic acid being lower than the corresponding values for tartaric acid, we can here of course employ the reduced formulæ IV and X without the necessity of resorting to the far more complicated V and IX; only in the case of No. 10 is, as will be seen from the table, any serious error incurred by using the reduced formula. As shown on p. 97 however, in dealing with tartaric acid, b is, in cases where the reduced formula can be applied, not dependent upon the absolute concentrations of A, B and C save inasmuch as the degree of dissociation of the salts will stand in a certain relation to these, but is, on the other hand, dependent upon the proportion between the values in question, and as O is also, as we have seen, dependent hereupon, b can therefore easily be taken as in relation to O and the proper degrees of dissociation of the salts. In Table 21, the concentration of hydrogen ions has been thus calculated for solutions of acid salt and normal malate, and of acid salt and malic acid, the relative values for acid, acid salt and normal salt, and

TABLE 22 b.

Empiric values for concentration of hydrogen ions in mixtures of sodium malate and malic acid. All solutions are $^{mol}/_{20} = {}^{n}/_{20}$ as regards sodium content; 100 cc. contains 5.0 mg. equiv. sodium. Cf. curves II a og b, Fig. 15.

No.	100 cc. solution contained			Molal concentration			Titration figures in cc. $^{n}/_{5}$ per 100 cc. solution	O = mg. equiv. titratable acid : mg. equiv. base	pH
	mol. malic acid cc.	mol. NaH malate cc.	mol. Na_2 malate cc.	A acid	B acid salt	C normal salt			
1	—	0.25	2.375	—	0.0025	0.02375	1.25	0.05	5.73
2	—	0.50	2.250	—	0.0050	0.02250	2.50	0.10	5.44
3	—	1.00	2.000	—	0.0100	0.02000	5.00	0.20	5.11
4	—	1.50	1.750	—	0.0150	0.01750	7.50	0.30	4.89
5	—	2.00	1.500	—	0.020	0.01500	10.00	0.40	4.72
6	—	2.50	1.250	—	0.025	0.01250	12.50	0.50	4.58
7	—	3.00	1.000	—	0.030	0.01000	15.00	0.60	4.44
8	—	4.00	0.500	—	0.040	0.00500	20.00	0.80	4.23
9	—	5.00	—	—	0.050	—	25.00	1.00	4.07
10	0.50	5.00	—	0.005	0.050	—	30.00	1.20	3.92
11	1.50	5.00	—	0.015	0.050	—	40.00	1.60	3.71
12	2.50	5.00	—	0.025	0.050	—	50.00	2.00	3.54
13	3.50	5.00	—	0.035	0.050	—	60.00	2.40	3.43
14	4.50	5.00	—	0.045	0.050	—	70.00	2.80	3.32

thus also for O being given; by drawing up the calculations, which have been made with different values for the degree of dissociation of the salts, in this manner, we avoid placing the various values for degree of dissociation in definite relation to the absolute concentrations of the substances present in the solutions, which is a point of importance, since the salts of the different bases have different degrees of dissociation at the same concentration. The results are shown in a co-ordinate system as a group of curves, each curve corresponding to its own degree of dissociation.

With regard to tartaric acid, it was mentioned that the curves obtained by calculation of the concentration of hydrogen ions in the different mixtures suggest that the formulæ are no longer valid when dealing with solutions which approach the pure acid salt. In the case of oxalic acid, this is not immediately apparent to such a noticeable degree, owing to the great difference between the two dissociation constants of this acid. With malic acid, on the other hand, the two calculated curves suggest that the formulæ

TABLE 23 a.

All solutions are $^{mol}/_{80} = {}^{n}/_{40}$ as regards calcium content. 100 cc. contains 2.5 mg. equiv. calcium. Cf. curves III a og b, Fig. 15.

No.	100 cc. solution contains			Molal concentration			Titration figures in cc. $^{n}/_{5}$ per 100 cc. solution	O = mg. equiv. acid: mg. equiv. base	pH
	mol. malic acid cc.	mol. $Ca^{1}/_{2}H$ malate cc.	mol. Ca malate cc.	A acid	B acid salt	C normal salt			
1	—	0.125	1.1875	—	0.00125	0.011875	0.625	0.05	5.57
2	—	0.250	1.125	—	0.0025	0.01125	1.250	0.10	5.26
3	—	0.500	1.000	—	0.0050	0.01000	2.500	0.20	4.91
4	—	0.750	0.875	—	0.0075	0.00875	3.750	0.30	4.71
5	—	1.000	0.750	—	0.0100	0.00750	5.000	0.40	4.53
6	—	1.250	0.625	—	0.0125	0.00625	6.250	0.50	4.41
7	—	1.500	0.500	—	0.0150	0.00500	7.500	0.60	4.29
8	—	2.000	0.250	—	0.0200	0.00250	10.000	0.80	4.11
9	—	2.500	—	—	0.0250	—	12.500	1.00	3.96
10	0.25	2.500	—	0.0025	0.0250	—	15.000	1.20	3.80
11	0.75	2.500	—	0.0075	0.0250	—	20.000	1.60	3.61
12	1.25	2.500	—	0.0125	0.0250	—	25.000	2.00	3.47
13	1.75	2.500	—	0.0175	0.0250	—	30.000	2.40	3.40
14	2.25	2.500	—	0.0225	0.0250	—	35.000	2.80	3.30

cannot be employed in this field. The reason of this it is, as already mentioned, impossible to determine, but it would seem likely that in solutions of the pure acid salt, molecular rearrangements *inter alia* take place, with formation of acid and the corresponding quantity of normal salt, which features have not been allowed for in the formulæ.

In setting the formulæ for the dibasic acids, it was taken for granted that the acid was combined with a monovalent base; where it had a divalent base, such as calcium hydroxide — which is exactly what we have to deal with in the cell-sap of succulent plants — the question is perhaps even more complicated, as we have the possibility of further combinations, both dissociated and non-dissociated, and there may thus be more than one acid salt present, as the calcium atoms can unite the malic acid anions in pairs, and the simplest acid salt, for instance $Ca_2H_2Ac_2$, might on dissociation be imagined as giving rise to an ion ($CaHAc_2$) which has no equivalent in the case of salts of the monovalent bases.

In order to investigate how far the calculated curves answer to actual conditions, both for malic acid in combination with a

TABLE 23 b.

All solutions are $^{mol}/_{40} = {}^{n}/_{20}$ as regards calcium content. 100 cc. contains 5 mg. equiv. calcium. Cf. curves IV a og b, Fig. 15.

No.	100 cc. solution contains			Molal concentration			Titration figures in cc. $^{n}/_{5}$ per 100 cc. solution	O = mg. equiv. acid: mg. equiv. base	pH
	mol. malic acid cc.	mol. $Ca^{1}/_{2}$ H malate[1] cc.	mol. Ca malate cc.	A acid	B acid salt	C normal salt			
1	—	0.25	2.375	—	0.0025	0.02375	1.25	0.05	5.46
2	—	0.50	2.250	—	0.0050	0.0225	2.50	0.10	5.15
3	—	1.00	2.000	—	0.0100	0.0200	5.00	0.20	(4.85)
4	—	1.50	1.750	—	0.0150	0.0175	7.50	0.30	4.60
5	—	2.00	1.500	—	0.0200	0.0150	10.00	0.40	4.42
6	—	2.50	1.250	—	0.0250	0.0125	12.50	0.50	4.29
7	—	3.00	1.000	—	0.0300	0.0100	15.00	0.60	4.18
8	—	4.00	0.500	—	0.0400	0.0050	20.00	0.80	4.00
9	—	5.00	—	—	0.0500	—	25.00	1.00	3.84
10	0.50	5.00	—	0.005	0.0500	—	30.00	1.20	3.74
11	1.50	5.00	—	0.015	0.0500	—	40.00	1.60	3.55
12	2.50	5.00	—	0.025	0.0500	—	50.00	2.00	3.44
13	3.50	5.00	—	0.035	0.0500	—	60.00	2.40	3.34
14	4.50	5.00	—	0.045	0.0500	—	70.00	2.80	3.23

monovalent and for the same with a divalent base, measurements were made with series of solutions, on the one hand of malic acid with sodium malate, and on the other with malic acid and calcium malate. In both cases, two series were measured; in each series of either the quantity of base was kept constant, this being 2.5 and 5 mg equivalent respectively per 100 cc. solution. The results are shown in Tables 22 a, b, and 23 a, b; the corresponding curves in Fig. 15.

Firstly, as regards the curves for sodium malate, it will be seen that they lie side by side, the difference between the p_H values in the separate ordinates being nearly alike throughout, varying between 0.08 and 0.05 (vide Table 24); in a single instance, however, 0.04 (answering to O = 1.60). On the whole, the differences are somewhat higher in the case of the two curves (I a and II a) for solutions of acid salt and normal salt viz: 0.08—0.06), than for the two (I b and II b) for solutions of acid salt and acid, where the differences lie between 0.06 and 0.05—0.04.

[1]) For better comparison of the acid salt and normal salt, I have throughout taken the composition of the former as $Ca^{1}/_{2}H(C_4H_4O_5)$, so that molecular solutions of the two salts contain like quantities of malic acid.

The difference between the two groups of curves in this respect is, however, so slight as to fall within the limit of experimental error; it is nevertheless probably real, as the corresponding curves for malic acid with its calcium salts reveal a greater difference

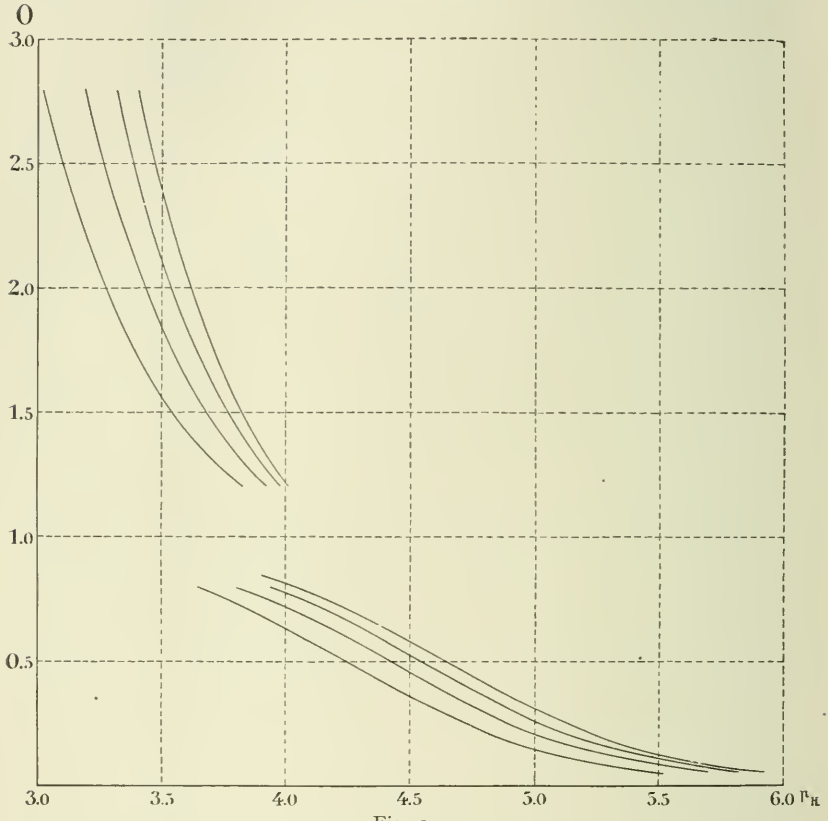

Fig. 14.

between curves III a and IV a (c. 0.11) than between II b and IV b, where the difference is c. 0.06. This of course stands in connection with the different values for degree of dissociation of the salts. As regards the course of the natrium malate curves compared one with another, it will be seen that the formulæ drawn up are applicable to the same extent in the case of salts of dibasic acids with divalent base and of salts of monovalent base. That the calcium malate curves show a higher degree of acidity than the corresponding curves for sodium malate is of course due to the fact that the calcium salts are not nearly so highly dissociated as the sodium salts.

A comparison between the curves thus found by experiment, and the calculated curves recently referred to shows, as will be seen from Figs. 14 and 15, that they do not entirely agree. In cases of solutions containing large quantities of acid salt as com-

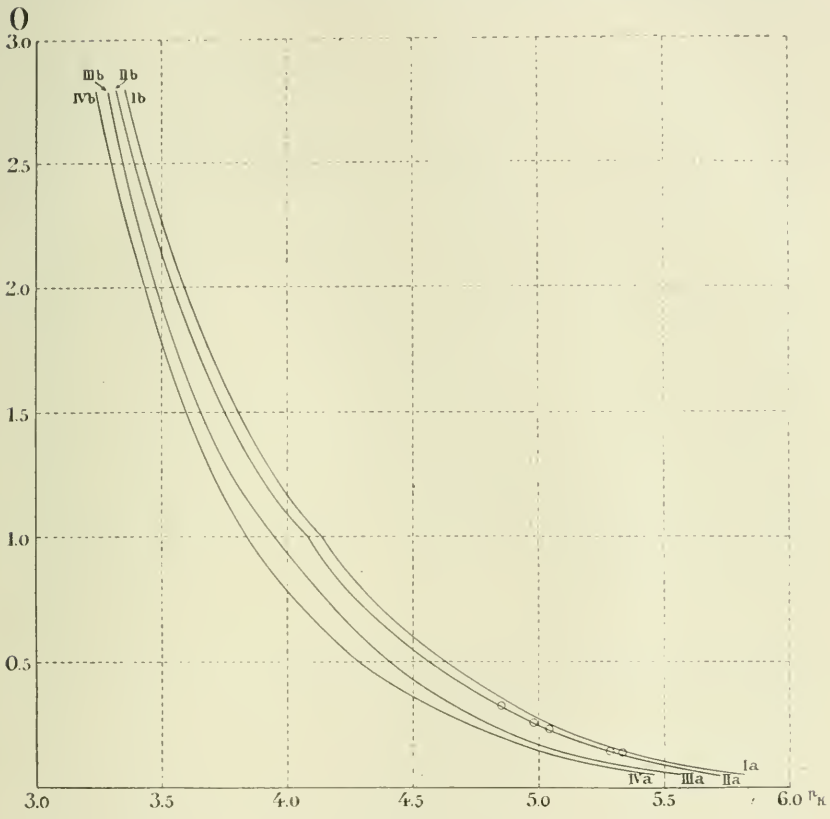

Fig. 15.

pared with normal salt, or large quantities of acid salt compared with free acid, thus approaching solutions of pure acid salt, the calculated curves cannot be used, as the formulæ employed do not cover the actual conditions. In those cases, however, where normal salt is found in abundance beside the acid salt, or where free acid is abundant as compared with acid salt, the theoretic curves reveal the same features as those found by experiment.

The next question to be dealt with is whether the conditions existing in artificially prepared mixtures of malic acid and malates really correspond to those in the natural sap. This will be treated in the following chapter; it may here be mentioned, how-

ever, that the sap itself is highly complicated, the malic acid being here found, not in combination with a single base, as in the artificial mixtures measured, but with a whole series of such; bases of potassium, sodium, calcium, magnium, iron and aluminium.

(In calculating the second dissociation constant of malic acid, k_2, we took β, i. e. the degree of dissociation of the second sodium atom in sodium malate, as c. 0.75—0.80 at a concentration of $^{mol}/_{32} = {}^{n}/_{16}$, and the degree of dissociation as c. 0.80 to 0.85 at a concentration of $^{mol}/_{64} = {}^{n}/_{32}$. By inserting the values for k_2 obtained in calculating p_H (vide Table 19 p. 86) in the last empirically obtained curves for sodium malate solutions, with p_H as usual representing the abscissa and O the ordinate, we find that the two series of measurements made at different times agree well together, the first four of the points answering to solutions $^{n}/_{19}$—$^{n}/_{21}$ as regards sodium content, coinciding with or lying close to the curves for solutions $^{n}/_{20}$ as regards quantity of sodium, while the 5th point, for which the solution is $^{n}/_{37}$ as regards quantity of base, falls between the two curves for solutions of $^{n}/_{20}$ and $^{n}/_{40}$ respectively as regards quantity of sodium, and nearest the latter.)

d. Comparison of measurements made with sap and those made with solutions of malic acid and its salts.

a. Rochea falcata D. C.

The theoretic observations above referred to in connection with the empiric results as to concentration of hydrogen ions in solutions of malic acid and its salts furnish a basis for comprehension of the conditions in the sap, further elucidated by experiments with Rochea falcata, of which plant an abundant and uniform material was obtainable.

As already mentioned, the juice of this plant contains malic acid and various salts of the same together with traces of hydrochloric, sulphuric, phosphoric, and possibly some very slight indication of citric acid. From the ash analyses it was found that the various bases occur in more or less constant quantities, albeit there will of course in this — as in all biological respects — be individual variations. The first question then will be, whether all these bases are associated with malic acid, or partly with

TABLE 24.

MALIC ACID WITH ITS SALTS.

O-values	Values for pH-determinations								
	a Theoretic values $\alpha=\beta=1$	b Sodium-malate solutions $n/_{40}$	c Sodium-malate solutions $n/_{40}$	d Calcium-malate solutions $n/_{40}$	e Calcium-malate solutions $n/_{20}$	b ÷ c	d ÷ e	b ÷ d	c ÷ e
0.05	5.92	5.81	5.73	5.57	5.46	0.08	0.11	0.24	0.27
0.10	5.59	5.52	5.44	5.26	5.15	0.08	0.11	0.26	0.29
0.20	5.24	5.18	5.11	4.91	(4.85)	0.07	(0.06)	0.27	0.26
0.30	5.01	4.95	4.89	4.71	4.60	0.06	0.11	0.24	0.29
0.40	4.82	4.79	4.72	4.53	4.42	0.07	0.11	0.26	0.30
0.50	4.64	4.64	4.58	4.41	4.29	0.06	0.12	0.23	0.29
0.60	4.46	4.50	4.44	4.29	4.18	0.06	0.11	0.21	0.26
0.80	4.05	4.29	4.23	4.11	4.00	0.06	0.11	0.18	0.23
1.00	—	4.13	4.07	3.96	3.84	0.06	0.12	0.17	0.23
1.20	4.01	3.97	3.92	3.80	3.74	0.05	0.06	0.17	0.18
1.60	3.78	3.75	3.71	3.61	3.55	0.04	0.06	0.16	0.16
2.00	3.62	3.59	3.54	3.47	3.44	0.05	0.03	0.12	0.10
2.40	3.50	3.48	3.43	3.40	3.34	0.05	0.06	0.08	0.09
2.80	3.41	3.38	3.32	3.30	3.23	0.06	0.07	0.08	0.09

other substances. In order to ascertain this, the following experiment was made:

Dialysis experiment with Rochea sap.

From 5 similar plants of Rochea, such leaves as were of more or less uniform size were taken (the uppermost, undeveloped leaves not used) amounting in all to 1519 g., and the sap extracted. Electrometric measurement of the sap showed:

	Hasselbalch's method	Hydrogen current method
p_H	4.351	4.356

Titration showed:

	I.		II.	
	30.41 g. sap		30.39 g. sap	
	Litmus cm^3 $n/_5$	Phenolpht. cm^3 $n/_5$	Litmus cm^3 $n/_5$	Phenolpht. cm^3 $n/_5$
	10.66—10.88	19.86 (uncertain)	10.75—10.91	14.70 (uncertain)
per 100 g. sap:				
	35.05—35.78		35.37—35.90	
Mean values:				
	35.42	65.37	35.64	48.37

The figures show, as usual, that the titration to litmus point was more or less sharp, that to phenolphtaleïn point somewhat uncertain. The point in the co-ordinate system found by p_H measurement and litmus titration fitted in well with the Rochea curve (p. 41).

The quantity of nitrogen found was 15.10 mg. per 100 g. sap.

152.25 g. of sap was calcined in the usual manner and quantitative determination of the various bases[1]) made. The result showed per 100 g. sap:

SiO_2	$Al_2O_3 + Fe_2O_3$	CaO
0.0030 g.	0.0734 g. = 4.31 mg. equiv.	0.2331 g. = 8.31 mg. equiv.

$Mg_2P_2O_7$	Alkali sulphate
0.0890 g. = 1.60 mg. equiv.	0.5510 g.

K_2PtCl_6 K_2SO_4

1.0441 g. ~ 0.3747 g. = 4,30 mg. equiv.

and thence calculated 0.1763 g. Na_2SO_4 = 2.48 mg. equiv.

Of the sap thus examined, 300 cc. (= 304 55 g.) was placed in a dialysis apparatus[2]) consisting of a film of collodium moulded to the shape of a test tube, and set in a cylindrical receptacle filled with water. The apparatus was so arranged that the outer liquid could easily be changed, a current of water being drawn through by suction, the outer liquid being constantly in connection on the one side with a water sucking-pump, on the other with a receptacle filled with distilled water free from carbonic acid. The diminished pressure produced by the pump was kept constant at 15 cc. mercury by a mercury regulator.

The collodium film was easily permeable for salts, but practically impermeable for albumins, this having been tested by 48 hours' dialysis of a solution of albumin rich in ammonium sulphate, placed within the film.

The dialysis of the sap was commenced on the 21 Sept. 1915 and terminated on the 6th October, 15 days later. The outer liquid was daily changed with c. 1 l. water free from carbonic acid; every third day the portions of outer liquid removed

[1]) As a qualitative examination of the calcined sap exhibited a considerable sodium reaction, a quantitative separation was here made between potassium and sodium.

[2]) Medd. fra Carlsberg Lab. **12**. 25 (1916).

were poured into one, thus giving five in all, Y I to Y V, each of 3 l.

During the 15 days the experiment lasted, the inner liquid gradually became a dark reddish violet, somewhat clouded, and decreasing also to a slight volume, hardly 40 cc. A couple of days after the inner liquid had ceased to decrease, and when it had been found that the residue after evaporation from Y III and Y IV was very slight, the dialysis was closed, and the various portions were then analysed.

Y I:

The outer liquid from the first three days (Y I) was evaporated in a vacuum on a water bath to a volume of 250 cc. Of this, 20 cc. was used for titration.

litmus	phenolpht. ($p_H = 9.2$)	
5.89 cc. $^n/_5$	6.70 cc. $^n/_5$	(good colour change, distinct red)
73.63 — -	83.75 — -	for the total quantity of liquid.

Electrometric measurement showed $p_H = 4.390$.

220 cc. was used for determination of ash. The result found was, calculated for total quantity of liquid in Y I:

$Al_2O_3+Fe_2O_3$	CaO	$Mg_2P_2O_7$	K_2PtCl_6	K_2SO_4	Na_2SO_4
0.1742 g.	0.5461 g.	0.2033 g.	2.7079 ∾	0.9719 g.	0.4729 g.

Y II:

Total volume of liquid = 3100 cc.

Of this 100 cc. titrated:

litmus	phenolpht.
0.65 cc. $^n/_5$ (uncertain)	0.65 cc. $^n/_5$ (sharp titration).

For the total quantity of liquid: 20.15 — -

Electrometric measurement showed: $p_H = 5.362$.

Ash residue, calculated for total quantity of dialysate, showed the following:

$Al_2O_3+Fe_2O_3$	CaO	$Mg_2P_2O_7$	$K_2PtCl_6 \approx K_2SO_4$	Na_2SO_4
0.0309 g.	(0.11—0.13 g.?)[1]	0.0478 g.	0.4419 g. ∾ 0.1586 g.	0.0802 g.

Y III:

Total volume of outer liquid = 3543 cc.

Titration of 500 cc. showed:

phenolpht.	
0.54 cc. $^n/_5$	(sharp titration).

For the total quantity of liquid: 3.83 — -

Titration to litmus point could unfortunately not be carried out.

The electrometric measurement of hydrogen ions, which showed a value for p_H of 8.23—8.28, was very difficult, and also valueless.

3000 cc. was evaporated and dried at 100° to a constant weight of 0.1212 g. corresponding to 0.1431 g. for the total quantity of liquid in Y III. The residue was then burned over a Bunsen burner to a constant weight of 0.0536 g. answering to 0.0633 g. for the total quantity of liquid.

Y IV:

Total volume of outer liquid = 2908 cc.

Titration of 500 cc. showed:

	phenolpht.
	0.25 cc. n/5
In the total quantity of liquid:	1.46 — "

Titration to litmus point could not be carried out.

Measurement of hydrogen ions was here, as in the case of Y III, very difficult. It showed: $p_H = 7.46—7.51$.

2378 cc. liquid was evaporated and dried at 100° to constant weight, and then calcined over a Bunsen burner to constant weight. The result showed:

	Dried evapor. residue	Ash residue
	0.0293 g.	0.0138 g.
For total Y IV:	0.0316 g.	0.0169 g.

Y V:

Total volume of outer liquid: 4037 cc.

Of this, 500 cc. used for titration:

	phenolpht.
	0.22 cc. n/5
For total quantity of liquid:	1.78 — "

The litmus paper was here likewise useless in titration.

Electrometric measurement, here likewise difficult, gave a result for p_H of 5.25—5.41.

3500 cc. was evaporated, dried to constant weight at 100° and calcined.

The result was as follows:

	Dried evaporated residue	Ash residue
	0.0153 g.	0.0073 g.
For total quantity of liquid:	0.0176 g.	0.0084 g.

Table 25.

SHOWING RESULTS OF THE DIALYSIS EXPERIMENT.

	mg. N.	Titration values in cc. $^n/_5$		Weight of dried evapor. residue	Weight of ash residue	$Al_2O_3 + Fe_2O_3$		CaO		$Mg_2P_2O_7$		K_2SO_4		Na_2SO_4	
		litmus	phenol-phta-leïn	g.	g.	g.	%	g.	%	g.	%	g.	%	g.	%
i 304.55 g. sap . .	45.99	107.89	—	—	—	0.2235	100.00	0.7099	100.00	0.2710	100.00	1.1412	100.00	0.5369	100.00
i Y. I	—	73.63	83.75	5.4185	—	0.1742	77.94	0.5461	76.93	0.2033	75.02	0.9719	85.16	0.4729	88.08
i Y. II.	—	20.15	20.15	1.1564	—	0.0309	13.83	(0.11—0.13)	ca. 0.17	0.0478	17.64	0.1586	13.90	0.0802	14.94
i Y. III	—	—	3.83	0.1431	0.0633	—	—	—	—	—	—	—	—	—	—
i Y. IV	—	—	1.46	0.0316	0.0169	—	—	—	—	—	—	—	—	—	—
i Y. V.	—	—	1.78	0.0176	0.0084	—	—	—	—	—	—	—	—	—	—
Inner liquid	2.3	2.25	—	1.18	0.0179	—	—	—	—	—	—	—	—	—	—

Inner liquid:

The dark-coloured inner liquid, which hardly amounted to 40 cc., was poured over into a 250 cc. measuring flask, the collodium tube was washed as thorougly as possible in distilled water, which was then added to the inner liquid, and finally, the retort was filled to the mark with distilled water. After shaking, 50 cc. was taken out for titration:

	litmus
	0.45 cc. $^{n}/_{5}$ (uncertain)
In total quantity of inner liquid:	2.25 —

Titration to phenolphtaleïn point proved impracticable, owing to the dark colour of the liquid.

The quantity of nitrogen in 25 cc. was found to be 0.23 mg

In the total quantity of inner liquid, there was 2.3 mg. N. The quantity of N dialysed out was thus $45.99 \div 2.3 = 43.69$ mg.

The electrometrical measurement of concentration of hydrogen ions, which proved not a little difficult, showed $p_H = 4.71 - 4.80$.

165 cc. of the inner liquid was evaporated, dried to constant weight at 100° and finally calcined. The result showed:

	Dried evaporated residue	Ash residue
	0.78 g.	0.0118 g.
For total quantity of inner liquid:	1.18 g.	0.0179 g.

The ash residue was strongly coloured with the characteristic ferric oxide tinge, and consisted possibly exclusively of this substance. That the iron had for the most part remained in the inner liquid without being dialysed out may be explained in two ways: either it is combined with organic substances which are not dialysable, or it is combined in salts, which are dissociated in acid and ferric hydroxide, of which the former constituent perhaps will dialyse out, while the ferric hydroxide passes over into colloidal form.

From the dialysis experiment, it appears that practically the whole quantity of base was dialysed out. The alkaline salts had entirely disappeared after the lapse of 6 days, by which time also the greater part of the remaining bases had likewise gone. The iron however, is here excepted, by far the larger part of this being probably retained. The titratable acid is also practically dialysed out. As to what kind of acid it is which finally

occurs in the inner liquid, this has not been determined, but the point will be referred to later on (p. 106 ff.).

From the foregoing, it must be presumed that practically the whole quantity of base — with the exception of the slight amount of iron which occurs in the sap — is combined with the malic acid, and the question then is, whether the malic acid and its salts are alone responsible for the relation between the litmus titration figures and the values for concentration of hydrogen ions, or whether other substances also have to be considered. In order to ascertain how far the former is the case, two methods suggest themselves: the purely theoretical, and the empirical. The theoretical method, however, is here impracticable, as in calculating the curves for the conditions just referred to, we should require to know, not only the amount of the different bases in the sap, but also the degrees of dissociation for the different salts at the concentrations in question, which last factor is unknown. The question may, however, be dealt with empirically, by titration and measurement of concentration of hydrogen ions in mixtures containing varying quantities of malic acid and malates in just that proportion in which they occur in the sap, the results being set out as usual in a co-ordinate system. The curve thus found should then answer to that found for the sap.

Such an experiment was carried out in the case of Rochea falcata. First of all, however, it will be well to describe the preparation of the mixture of malates answering to the sap of this plant.

From the ash analyses of Rochea sap given in Table 7 b (p. 38) and the similar analyses recently referred to (p. 98), the following mean values were found for the different bases per 100 g. sap.

Al_2O_3	CaO	$Mg_2P_2O_7$	K_2SO_4	Na_2SO_4
4.47	8.48	1.33	4.30	2.48 mg. equiv.

It was thus required to prepare solutions containing malates with bases in the proportions above given. In the case of the aluminium salt, the method employed was as follows: A weighed quantity of a salt of ammonia alum, which had been recrystallised several times, was dissolved in water, an abundant amount of ammonium chloride and ammonia was added until the liquid

became ammoniated, when it was heated to boiling point. The aluminium hydroxide given off was filtered out and well washed in hot water, after which it was removed from the filter, dissolved in a small surplus of the calculated quantity of acid, and aluminium hydroxide precipitated anew after the addition of ammonium chloride with ammonia. The precipitate filtered off was then washed in water, until a small sample dissolved in sodium hydroxide gave neither sulphuric acid nor hydrochloric acid reaction. The aluminium hydroxide thus cleansed was then transferred to a large flask, previously well cleaned by being subjected to a through current of boiling steam for some twenty minutes. A calculated quantity of potassium hydroxide was then added, and the flask closed with a cork pierced with a glass tube bent to a right angle. The liquid was now boiled until solution was complete, and then slightly cooled, the glass tube having been previously connected up to a tube of soda-lime to prevent any carbonic acid from penetrating in to the liquid. After partial cooling, the liquid was filtered over into a measuring flask, containing a quantity of malic acid slightly in excess of that corresponding to the presumable amount of aluminium hydroxide and potassium hydroxide. During filtration, the filtrate was well mixed, by careful shaking, with the malic acid, and after the liquid had been fully cooled, the retort was filled with boiled water up to the mark. A suitable quantity was then removed and used for quantitative determination of aluminium as Al_2O_3 and potassium as K_2SO_4. We had now a solution with known content of aluminium, potassium and malic acid, and a quantity of the solution containing the required amount of aluminium was employed. To this quantity of liquid, a calculated amount of malic acid was then added, with the amount of potassium hydroxide lacking. The potassium hydroxide used was a preparation from Merck, guaranteed free from Al_2O_3, SiO_2, H_2SO_4

The calcium malate was prepared from equivalent quantities of malic acid and a clear filtered solution of calcium hydroxide as previously described. The magnium malate was prepared with a pure preparation of magnium oxide from Kahlbaum, which was dissolved in a small surplus of malic acid while heating in a boiling water bath.

Calculated quantities of all these solutions were poured to-

gether and evaporated in a porcelain dish on an electric water bath to a volume slightly below that desired. The liquid was then transferred to the measuring flask, and finally, the requisite quantity of sodium was added, in the form of the $^n/_5$ solution of sodium hydroxide mentioned on p. 82. The retort was then filled with boiled water up to the mark.

In this manner, two solutions were obtained, which were uniform as regards quantity of base, the one, however, (I) slightly richer in malic acid than the other (II), the former containing somewhat more than the quantity of malic acid equivalent to the bases, whereas the other contained malic acid equivalent to abt. 60 % of the quantity of aluminium and the total quantity of the remaining bases. In regard to concentration, these two solutions were so composed that they should, on addition of malic acid and water, be diluted in the proportion of 20 to 25.

The following table shows the composition of the different mixtures per 109 cc. solution[1]), the titration figures for the same, likewise per 100 cc., and the sum of the titration figures for the salt solution and the weighed quantity of acid added (column h). If the titrations could be carried out with perfect accuracy, the figures in e should be equal to the corresponding values in h; this, however, is not the case, but a comparison of the two columns will give an idea as to the degree of accuracy obtained in titration. In column g will be found the figures for difference between titration to litmus and to phenolphtaleïn point, which should all be alike if the titration were sharp; these also, however, indicate the inaccuracy attaching to the method. Finally, in i, we have the values found by electrometric methods for the concentration of hydrogen ions in the mixtures. The results are drawn with △ points in the coordinate system (Fig. 7) on p. 42, which already presents a view of the conditions in sap of Rochea falcata. The curve representing the artificial mixtures is drawn with a dotted line, the Rochea curve being fully drawn. As will be seen from the figure, these two curves very nearly coincide, lying side by side, and with only very slight distance between; they furnish thus a proof that in the sap of Rochea falcata, the determining factor for concentration of hydrogen

[1]) 25 cc. of each mixture was prepared. 10 cc. of which was used for titration with $^n/_{10}$ sodium hydroxide.

TABLE 26.

a	b	c	d		e	f	g	h	i
No.	No. of salt solution	cc. acid added per 100 cc. solution	Titration value of salt solution in cc. $^n/_5$ per 100 cc. complete solution		Titration figures in cc. $^n/_5$ per 100 cc. solution		Phenolphtaleïn ÷ litmus	Litmus figures for salt solution + added acid in cc. $^n/_5$ per 100 cc. solution	pH
			litmus	phenol-phtaleïn	litmus	phenol-phtaleïn			
1	II	0	2.88	13.96	2.88	13.96	11.08	2.88	5.595
2		4.12 $^n/_5$			8.60	18.80	10.20	7.00	5.195
3		7.16 -			—	—	—	10.04	5.002
4		9.96 -			14.40	24.20	9.80	12.84	4.879
5	I	0	14.85	26.30	14.85	26.30	11.45	14 85	4.863
6		2.48 -			16.85	27.50	10.65	17.33	4.778
7		7.48 -			22.30	33.50	11.20	22.33	4.619
8		12.48 -			26.65	36.85	10.20	27.33	4.498
9		17.48 -			31.43	42.63	11.20	32.33	4.378
10		4.48 $^n/_1$			36.25	48.00	11.75	37.25	4.305
11		5.44 -			41.10	53.00	11.90	42.05	4.200
12		6.52 -			47.25	57.55	10.30	47.45	4.145
13		7.48 -			53.30	63.30	10.00	52.25	4.063
14		8.40 -			57.75	69.80	12.05	56.85	4.005
15		9.44 -			62.70	73.15	10.45	62.05	3.949

ions is the quantity of malates contained in the sap, together with the amount of titratable malic acid. That the two curves should exactly coincide was naturally not to be expected; it must be remembered, in the first place, that the titrations themselves are subject to a certain degree of inaccuracy, the extent of which, in the case of artificial salt solutions, will be apparent from the foregoing; it is, as we have likewise seen, somewhat higher for the actual sap And in the second place, the electrometric measurements are likewise subject to a certain degree of error, which, in the case of the artificial solutions, doubtless does not exceed 1 millivolt, equivalent to abt. 0.02 in p_H, and in the sap, probably somewhat more than this. Last but by no means least, the great variability of the material must be taken into consideration. This is directly illustrated by the points in the co-ordinate system in which the Rochea curve is drawn, as also by the values given in Table 7 for the quantities of the different bases, from the averages of which the artificial mixtures employed were composed. It should further be borne in mind, that the sap has been found to contain traces of hydro-

chloric acid, sulphuric acid and phosphoric acid, which substances are not included in the artificial mixtures.

Finally, mention may be made of some experiments carried out with the artificial salt mixtures and those portions of the sap which could not be dialysed out, as various points would seem to suggest that it is these which — as already mentioned —, are partly responsible for the difference in the figures for titration to litmus and to phenolphtaleïn point; these likewise, which give rise to the change of colour in the sap, and partly also to the marked precipitation during titration. 300 cc. of sap was dialysed — in the manner previously described —, until the volume did not further decrease. The dialysis was commenced on the 11th January 1916, and the volume of the sap decreased continually until 31 January, after which no further decrease was observable. On the 3rd of February therefore, it was removed from the collodium tube, which was afterwards rinsed with a small quantity of water. A total volume of 30 cc. was obtained. This solution would, when diluted to tenfold volume, answer to the original volume of the sap. It was used partly for titration and measurement of concentration of hydrogen ions, partly for addition to the artificial mixtures, titration and measurement of the latter being thereafter made. The results are shown in Table 27, in explanation of which the following should be noted: The experiments are so numbered as to show which of them correspond to the experiments in the foregoing series, the two series differing only in the fact that the mixtures of the last had added to them some of that portion of the sap which remained over after dialysis.

The prepared solutions 1 and 2 had a volume of 25 cc. each; the 4 next of 50 cc. each. Finally, solution a consisted exclusively of 2.5 cc. of the mentioned portion of sap + water to a total volume of 25 cc.

The results of the experiments show the following:

The non-dialysable portion of the sap does not interfere with the concentration of hydrogen ions in the artifical mixtures, as long as we have to deal with mixtures where the concentration of hydrogen ions is over $10 \div 4.7$. From Exp. 6 it will be seen that in this case, with a concentration of hydrogen ions of $10 \div 4.77^8$, a slight dislocation takes place in the acid direction, viz; to

Table 27.

No.	No. of salt solution	cc. of salt solution + cc. of dialysis liquid	Acid added, cc.	Volume of liquid titrated	Direct titration figures in cc. n/10 to litmus	Titration figures in cc. n/5 per 100 cc. solution to litmus	pH
1	II	20 + 2.5	0	10	1.90	9.50	5.244
2	II	20 + 2.5	1.03 n/5	10	2.92	14.60	5.035
6	I	40 + 5.0	1.25 -	25	11.48	22.96	4.739
8	I	40 + 5.0	1.23 n/1	20	13.05	32.62	4.489
10	I	40 + 5.0	2.28 -	10	8.50	42.50	4.283
13	I	40 + 5.0	3.77 -	10	11.60	58.00	4.071
a		0 + 2.5	0	15	0.30	1.00	4.72—5.00

$10 \div 4.739$, and at lower concentrations, a greater alteration is found This shows, that the non-dialysable part of the sap contains substances which appear as acids at concentrations of hydrogen ions below $10 \div 4.77$, which also appears from the measurements made with the sap diluted with water (Exp. a).

As regards titration figures for the mentioned portion of the sap, it should first be observed that the colour of the sap was so dark that titration to phenolphtaleïn point proved impracticable. On titration to litmus point, the quantity consumed per 100 cc. solution was 1.00 cc. n/5. We might therefore expect that it would be possible, from the titration figures in Table 26, to arrive by calculation at the corresponding values in Table 27; this, however, proved not to be the case, the latter figures being higher. The non-dialysable portion of the sap thus contains substances which in conjunction with salts of polyvalent bases give a higher titration value than the sum of the titration figures for the separate solutions. This is, however, not altogether without parallel, as something similar is known to be the case for instance with phosphoric acid, which in the presence of calcium may be titrated as a tribasic acid, while without calcium, it may be tritrated as a dibasic, when sodium hydroxide and phenophtaleïn are used in titration. We have probably therefore, in the case of the sap, to deal with a substance having the character of a polybasic acid. That even the non-dialysable parts of the sap have undergone certain changes during dialysis appears not only from the marked alteration in colour, but also from the fact that

the addition of this portion of the sap to the salt solution brought about a precipitation, which was immediate and abundant at the low concentrations of $10 \div 5.244$ and $10 \div 5.035$, and after some hours, also in the remaining solutions, but to a slighter degree, and more slowly, as the concentration of hydrogen ions in the liquid increased.

β. Cotyledon obvallata Haw.

In the case of Cotyledon obvallata, determinations were made, as already mentioned, of the quantity of bases in the sap, and with the aid of these, it was possible to carry out experiments similar to those just described for Rochea falcata. In Cotyledon. however, the determinations of ash are far less numerous, and there would seem to be a greater variability in the quantity of base, which of course may occasion a lesser degree of agreement in the results obtained.

Only in the case of Experiments 7, 8 and 9 in Table 4 b are complete analyses of ash components available, the quantity of potassium not having been determined in the first six. And it would now seem that there may have been a not altogether inconsiderable difference between the plant material utilised for the first six experiments and that used for the last ones, as the first six contained far less aluminium, with, on the other hand, a good deal more calcium and magnium, while among the last three experiments again, No. 9 appears to have an abnormally high ash content. In Nos. 7 and 8, however, we have, besides complete determination of all bases, also determination of the titratable quantity of acid and of the concentration of hydrogen ions, and the figures for the bases obtained in these last two experiments have therefore been taken as the basis for a series of experiments with artificial mixtures of malic acid and malates in the same manner as recently described for the comparative experiments with Rochea sap.

A salt solution was then prepared, which, when diluted with varying quantities of malic acid and water in proportion 20 to 25 cc., contained, per 100 cc.:

4.26 mg. equiv. Al, 7.14 mg. equiv. Ca, 0.48 mg. equiv. Mg, and 2.16 mg. equiv. K, and in these mixtures, measurements of concentration of hydrogen ions and titrations were made. 25 cc. of each solution was prepared, and 15 cc. used for titration.

TABLE 28.

a	c	d		e	f	g	h	i
No.	cc. acid added per 100 cc. solution	Titration figures for salt solution in cc. n/5 per 100 cc. complete solution		Titration figures in cc. n/5 per 100 cc. solution		Phenolphtaleïn ÷ litmus	Litmus figures of salt solution + added acid in cc. n/5 per 100 cc. solution	pH
		litmus	phenol-phtaleïn	litmus	phenol-phtaleïn			
1	0	2.40	10.40	2.40	11.99	9.59	2.40	5.520
2	1.97 n/5			4.10	13.99	9.89	4.37	5.163
3	4.97 -			7.23	17.09	9.86	7.37	4.838
4	8.01 -			10.27	20.26	9.99	10.41	4.710
5	11.32 -			13.83	23.16	9.33	13.72	4.545
6	16.03 -			18.33	27.99	9.66	18.43	4.391
7	4.44 n/1			24.33	33.79	9.46	24.60	4.179
8	6.06 -			32.20	42.12	9.92	32.70	4.036

which was carried out with n/10 sodium hydroxide. The results are shown i Table 28, and drawn in a co-ordinate system Fig. 4, with △ points from which the dotted curve has been constructed. On comparing these results with those obtained from the sap, which latter are represented by the fully drawn curve, it will be seen that although the dotted curve does not coincide with that for the sap, a number of the points for sap nevertheless fall within the dotted line. It must therefore be supposed that the artificial salt mixture was somewhat richer in dissociated malates than was generally the case with the sap. The table also shows, as in the case of the artificial salt mixture corresponding to Rochea sap, that the titration to litmus and phenolphtaleïn points is subject to some degree of uncertainty, which will likewise be seen from the figures for difference in titration to the two stages. These figures for difference are here on the whole somewhat lower than in the case of the artificial Rochea mixtures, which agrees with the somewhat higher figures for aluminium in the latter. On comparing the figures for difference in titration (c. 9.7) for the artificial mixtures with those found in the plant experiments 7 and 8 Table 4 b, we find that the last-named, c. 6, is somewhat lower, despite the fact that the quantity of aluminium is the same; such difference may, however, easily be explained as due to the difficulty of titrating sap on account of its peculiar

colour and the alterable substances already mentioned as therein contained. These are, it is true, not found in anything like the same degree in Cotyledon obvallata as in Rochea, where they disturb the process of titration to phenolphtaleïn; they are, however, present to such a degree as to render it desirable to carry out the titration to this stage as rapidly as possible. On the other hand, aluminium malate requires — as already mentioned — a particularly slow titration in order to give the phenolphtaleïn red ($p_H = 9.2$).

e. Concluding remarks on the concentration of hydrogen ions and quantity of acid in the cell-sap of succulents.

In all the experiments with sap, it has been shown that the concentrations of hydrogen ions found lie within certain definite limits; the lowest of the values measured was $10^{\div 5.7}$, and the highest $10^{\div 3.9}$. As to how far there is any possibility that the concentration of hydrogen ions can fall lower than $10^{\div 5.7}$ I do not venture to say. On the one hand, the curves show, that a slight alteration in the quantity of titratable acid at low concentrations occasions a considerable change in the values of these; on the other hand, it should be noted that the quantities of acid corresponding to the lowest values found for p_H were relatively very low. The question now is, whether a further decomposition of the acid in the sap of succulents than that here observed can take place. This, it must be admitted, might well be imagined to be the case, as we have no sort of guarantee that the circumstances under which the present investigations were carried out did not of themselves involve certain conditions whereby the process of decomposition was restricted. It is by no means impossible, for instance, that the temperature failed to reach a height, or the light to attain an intensity at which further decomposition could otherwise take place. At the same time, however, it must be remembered, that even if a further decomposition of the malic acid could occur, there are also, in the sap of the plants investigated, various substances which at concentration of hydrogen ions below $10^{\div 6.81}$ appear as weak acids, and it is doubtless possible that some of them may already at a slightly more acid stage exhibit an acid character.

With regard to the upper limit also, of $10^{\div 3.9}$ we have here

again no guarantee that this is the highest possible. As already mentioned, the rich accumulation of acid which may take place in succulents is most marked after a period of intense light, during which the assimilation of carbonic acid has been particularly active so as to furnish an abundant supply of material for acid secretion, and it is possible that the climate of Denmark does not present the most favourable conditions in this respect. On the other hand, it will be seen from the curves that even a slight dislocation of the p_H values within such limits demands a very considerable increase in the amount of acid. Thus from curve IV b Fig. 15, it will be seen that a movement of p_H from 4.00 to 3.8 requires an increase of acid from $0.8 \cdot 5 \cdot 5 =$ 20 cc. $n/5$[1]) to $1.07 \cdot 5 \cdot 5 = 26.75$ cc. $n/5$, whereas a movement of p_H from 5.0 to 4.80 only demands an acid increase from $0.13 \cdot 5 \cdot 5 = 3.25$ to $0.21 \cdot 5 \cdot 5 = 5.25$ cc. $n/5$.

Finally, it should be mentioned that the sap of succulents will in all probability never contain acid in conjunction with acid salt, but will on the other hand, always exhibit varying quantities of acid salt and normal salt, as will be seen from the following:

In Rochea falcata, an average of 21 mg. equivalents base was found, that is to say, 21 mg. equivalents normal salt per 100 cc. sap, presuming the entire quantity of base to be combined with malic acid, which, from the results of the dialysis experiment, would seem to be the case. This would require 21 mg. equivalents titratable acid, i. e. 105 cc. $n/5$ acid per 100 g. sap, in order that the sap should contain exclusively acid salt, and an even greater quantity of acid to give acid salt in conjunction with acid. Nothing approaching such quantities of titratable acid has, however, been found in any instance on titration to the litmus point; the highest value obtained was abt. 60 cc. per 100 g. sap; a rise to 105 cc. could hardly occur, however favourable the general conditions might be for secretion of acid.

In Cotyledon obvallata, the quantity of base found was c. 14.8 mg. equivalents per 100 g. sap, answering to 14.8 mg. equiv. normal salt. If the sap were to contain exclusively acid salt, this would require $14.8 \cdot 5 = 74.0$ cc. $n/5$ titratable acid, whereas the highest value found was 22 cc. $n/5$.

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[1]) The quantity of acid in the ordinate height 0.8 is equal to $0.8 \cdot 5 \cdot 5$ cc. $n/5$, the ordinate height denoting the quantity of acid expressed in cc. $n/1$ per mg. equivalent base, here amounting to 5.

Finally, in the case of Cotyledon linguæfolia, the quantity of base found was on an average 16.6 mg. equiv. per 100 g. sap. The quantity of titratable acid required to give exclusively acid salt would here correspond to $16.6 \cdot 5 = 83.0$ cc. $^n/_5$.

In these two plants, then, there is equally little reason to suppose that free acid occurs together with acid salt, even though possibly part of the quantity of base may be combined with other substances than the malic acid.

We thus find, as one of the most important results of these investigations, that the concentration of hydrogen ions in the sap of Rochea falcata, Cotyledon obvallata and Cotyledon linguæfolia, probably also in all succulents, is determined by the proportion between the quantities of acid malate and normal present in the sap, this mixture acting in a very pronounced degree as a buffer. The term "buffer" ("tampon") was first introduced by Fernbach and Hubert[1]), to meet a special case; as already mentioned, however, it is in the works of S. P. L. Sörensen[2]) and Henderson[3]) that the great importance of such buffer mixtures for the organism was first thoroughly dealt with. In the living organism, there are doubtless a great number of different buffers; these may generally speaking be said to be but slightly dissociated substances, possessing a large supply of hydrogen or hydroxyl ions, which can be dissociated off in case of a decrease in the free hydrogen or hydroxyl ions of the substrate, thus preventing any sudden alteration in the concentration of hydrogen ions. The substances whose regulating faculty in this direction have hitherto been particularly observed are, phosphates, carbonates, proteïns and decomposition products of the same. To these should now be added malate mixtures, which in the case of succulents exert the same effect as the substances already named in animal organisms. The substances which occasion difference in the titration figures for phenolphtaleïn and litmus will likewise be capable of acting as buffers and preventing the concentration of hydrogen ions from going over to the alkaline side, as at concentrations of hydrogen ions less than

[1]) Comptes rend. d. Laborat. d. Carlsb. **131**. 293 (1900).

[2]) Comptes rend. d. Laborat. d. Carlsb. **8**. 53 (1909); Ergebnisse der Physiologie. **12**. 525 ff. (1912).

[3]) Ergebnisse der Physiologie. **8**. 254 (1909).

$10^{\div 7}$ they appear in the form of weak acids; in addition, it is in many ways quite possible that other substances may be present which at a more acid stage could act as buffers. The question may of course be further investigated by titration at stages within concentration of hydrogen ions higher than $10^{\div 7}$.

C. EXPERIMENTS WITH NON-SUCCULENT PLANT ORGANS.

As mentioned in the Introduction, the concentration of hydrogen ions in a substrate where chemical processes take place is of decisive importance to the latter. It has frequently been shown, for instance, that this factor exerts a determining effect upon enzyme action, and that this must be the case will very readily be understood when we consider the new points of view from which the enzymatic processes are now discussed, as the enzymes — or the substances with which they are associated — must be regarded as amphoteric electrolytes, the degree of dissociation of which is determined by the concentration of hydrogen ions in the substrate. In certain cases, the enzymatic qualities have been found in the non-dissociated portions (e. g. in the case of invertin) while in others, the dissociated parts — and of these either the anion or the kation — were the active elements (in trypain, for instance, it is the anion which possesses the proteolytic quality). All this was first advanced by Michaëlis[1]) with regard to pepsin, and at the same time by Loeb[2]) for pepsin and trypsin, after it had been found that the various enzymes may either be absorbed by various absorbents in substrate of suitable reaction, or, when transferred to an electric field, exhibit definite migrations in the direction of the anode or katode, and that the direction of such migration may be altered by placing the enzyme in an acid or alkaline substrate instead of the neutral[3]). The theory has since been applied by various investigators to other enzymatic processes. For the decomposition of the enzyme also, the concentration of hydrogen ions plays an important part, as likewise in the case of the physico-chemical

[1]) Biochem. Zeits. **17**. 233 (1909).
[2]) *ibid.* **19**. 534 (1909).
[3]) Michaëlis, *ibid.* **16**. 81 and 486 (1909).

conditions in proteins[1]), all of which are of importance to the living organism.

Consequently, then, it will be necessary to know the true reaction in the living cells before we can arrive at a thorough comprehension of what takes place therein. In the case of the succulents, it was shown that the p_H values range from 3.9 to 5.7, and it was also mentioned as probable that the limits for these values would not be essentially different from these. It will naturally be interesting hereafter to consider, how the enzymes present in the leaves of succulents behave in the various concentrations of hydrogen ions which occur; this question, however, has not been touched upon in the present investigations.

The succulents must, as already mentioned, be regarded as occupying an extreme position among green plants, owing to the considerable variations which occur in the quantity of acid contained. The question then arises as to how other vegetable organs will appear from this point of view.

Some few measurements have therefore been made, on the one hand with seedlings of *Lupinus albus*, which might not unreasonably be supposed to resemble the majority of non-succulent plant organs in this respect, and on the other, with citrus fruits, as these might be supposed to exhibit extreme values for concentration of hydrogen ions. Finally also, with the same end in view, experiments were made with the pitcher-fluid of Nepenthes.

1. EXPERIMENTS WITH LUPINUS ALBUS L.

Seedlings were cultivated in flower pots filled with sawdust, placed in a south window of the library at the Carlsberg Laboratory, at ordinary indoor temperature.

The experiments were made with juice from the stalks and the first small leaves; only in E and F were the stalks alone employed. The plants in A were etiolated by being kept constantly under a black screen; those in F were placed in a horizontal position and subjected to geotropic stimulus for the last hour before crushing and analysis.

The results show that the quantity of acid measured on titration to litmus point appeared in the case of normal seedlings to increase somewhat with increasing age of the plant; the value

[1]) Ergebnisse der Physiologie **12**. 472 ff., 506 ff. (1912).

	a	b	c	d	e	f	g
				pH		Titration figures	
	No. of plants	Weight of plants	% dry matter	Lak-moïd-test	Electrom. measurem. H-current method	Litmus cc. n/5 per 100 g. sap	Phenolpht. cc. n/5 per 100 g. sap
A. Etiolated plants 19 days old	19	33.34	5.79	5.9	5.89	10.35	69.57
B. Normal plants 19 days old	30	43.27	8.09	—	5.78	3.65	86.88
C. Normal plants 20 days old	24	55.64	6.68	—	5.93	6.51	90.00
D. Normal plants 22 days old	24	49.17	—	—	6.03	7.48	106.31
E. Normal plants 14 days old	39	46.62	—	5.9	5.95	3.54	26.34
F. Plants subj. to geotropic stimulus; 14 days old	38	50.99	—	—	5.95	7.33	62.85

for the normal plants 14 days old cannot, however, be taken in direct comparison with the rest, as the leaves had in this case also been removed. Both in the etiolated and in those subjected to geotropic stimulus the quantity of acid rose beyond the normal[1]). None the less, the concentration of hydrogen ions remained nearly constant, the values for this lying between 5.78 and 6.03. In the first experiment, with etiolated plants as against normal, it will be seen that the highest quantity of acid is found together with the lowest concentration of hydrogen ions; otherwise, the slight differences in the values found for p_H are probably of a purely individual character. The difference between titration figures for litmus and phenolphtaleïn appears to increase with increasing age in the normal plants.

In the etiolated plants, on the other hand, we find a markedly inferior quantity of the substances which determine this distance, while the substances in question appear to increase in quantity on geotropic stimulus.

It would thus seem as if the concentration of hydrogen ions

[1]) Kraus has observed that the acids in etiolated plants often occur in quantities deviating from the normal: the quantity of acid is also affected during geotropic stimulus. Abhandl. d. Naturf. Ges. z. Halle. **15**. 23 (1880); **16**. 12 (1884).

in the stem and root is maintained within a very definite and very restricted field about $10^{\div 6}$. That this value remains more or less constant is not only shown by the experiments here carried out — which are, it must be admitted, but few in number — but is further supported by the fact that the investigations of aqueous extracts of malt at Carlsberg Laboratory have constantly shown the same values (viz, ranging from $10^{\div 5.7}$ to $10^{\div 6.1}$) whether the barley seedlings had germinated for a longer or shorter time; the values found for 6—8—10—11—13 days plants were the same as those for the soaked corn. The measurements of concentration of hydrogen ions in extract of malt is as a rule made by colorimetric methods, either with methyl red or with para-nitrophenol as indicator, but experiments by electrometric measurement have sometimes been made as a test. In the malt extract also, and in the extract from the soaked corn, we find a considerable distance between the titration figures for litmus and phenolphtaleïn points; thus in extract calculated to 25 g. dry matter, the values found were:

		litmus cc. $n/_{10}$	phenolphtaleïn cc. $n/_{10}$	phenolpht. ÷ litmus cc. $n/_{10}$	pH
I.	Soaked corn	4.03	11.76	7.73	5.7
II.	6 days old seedlings	5.53	23.09	17.56	5.9
III.	8 — —	5.38	23.79	18.41	5.9
IV.	11 — —	5.38	25.52	20.14	5.9

That the concentration of hydrogen ions in seedlings thus remains almost constant despite the variation — at times very considerable — in quantity of acid (as shown in the case of the Lupin plants) must here also be due to "buffer" effect.

2. EXPERIMENTS WITH CITRUS FRUITS.

The juice of the lemons was pressed out, centrifuged, and filtered; the results showed:

No.	pH	litmus	phenolpht.
1	2.19	527.7	540.0 cc. $n/_5$ per 100 g. sap
2	2.25	502.1	518.0 — · — — · —
3	2.24	536.0	539.0 — · — — · —

In a solution of citric acid, with titration figure 50.6 cc. $n/_5$ per 100 g., pH was found = 2.23, a value falling very close to

that found in the lemons, where the quantity of acid, however, was ten times as great. There must thus in the lemons be buffer elements present which urge the concentration of hydrogen ions over in an alkaline direction.

Whether it will be possible to find in other plant organs a concentration of hydrogen ions equal to or higher than that in the lemons, I do not venture to say, but it would seem hardly likely. The values are in themselves widely different from what is otherwise known in living tissue, and one is inclined to be surprised that such can occur at all. It should be borne in mind, however, that a full-sized, but not fully ripe lemon is really not an organ of ordinary vegetative character, but should more properly be regarded as a passive organ, in which light and high temperature probably for the most part complete the ripening process, after which the organ is doomed to decay. It would nevertheless be most interesting to investigate the conditions as they appear in fruits from the first stages of formation, while distinct growth processes are still predominant, and chemical processes also of various kinds may be still supposed to take place.

3. EXPERIMENTS WITH PITCHER-FLUID OF NEPENTHES.

For these experiments, three distinct species were employed, each of which was separately examined.

Several pitchers of Nepenthes Tiveyi were available, in one of them the sap was a muddy brown, with the wings and carapace of an insect, — the remainder of which, by the way, was entirely digested — floating therein. In the remaining pitchers, the juice was clear, with a number of small flies swimming in it. The juice of the first pitcher was kept separate (A), that from the remainder being taken together (B): In A, p_H was found by Hasselbalch's method = 5.87, but on subsequent treatment with hydrogen current the value of p_H rose continually. On the following day, when it had reached 8.15, the experiment was closed. In B, on the other hand, Hasselbalch's method showed 4.61, and the hydrogen current a constant value of 4.67; 5 cc. of B showed on titration an acid content equivalent to 0.06 cc. $n/5$.

In the juice of Nepenthes paradisa, a constant value of p_H = 3.20 was found. The juice here contained various undigested

or partially digested insects. 5 cc. showed on titration an acid content equivalent to 0.08 cc. $^n/_5$.

Finally, in a third species, (Nepenthes sp?) Hasselbalch's method showed $p_H = 5.51$, which value rose, on transfusion with hydrogen, to 7.97 in the course of 24 hours, when the experiment was terminated. 5 cc. of sap showed on titration 0.01 cc. $^n/_5$.

It will be seen from the foregoing, that the juice in calices irritated by insects shows a concentration of hydrogen ions above $10 \div 7$, thus here $10 \div 4.6$ and $10 \div 3.2$, whereas the juice in non-stimulated pitchers appears to be neutral. or possibly rather to have a fluctuating concentration of hydrogen ions. On titration, again, no acid was discerned in the latter case, whereas in the two previous experiments, the values obtained were sufficient to be admitted as real. This result agrees well with the observation made by Clautriau[1]), to the effect that stimulus of the pitchers not only occasions secretion of proteolytic enzyme, but also of acid.

D. DETERMINATION OF CONCENTRATION OF HYDROGEN IONS WITH LAKMOÏD PAPER.

We may now glance briefly at the manner in which the lakmoïd paper measurements appear when compared with the results obtained by the electrometric method. In the tables for plant analysis, two values are as a rule stated for the lakmoïd test, viz; that found prior to extraction of the sap, and that found after the electrometric measurement; also, in many cases, two values for the electrometric measurements, to wit, that obtained by the hydrogen current method and that given by Hasselbalch's method. In the accompanying table, only one value is shown for the lakmoïd test, and one for the electrometric measurement, the values here employed being throughout those showing the greatest difference. On comparing in this way results obtained by the two methods, we are on the safe side with regard to magnitude of the error attaching to the process of measurement with lakmoïd paper. The results for each plant are kept separate, and arranged in order of decreasing concentration of hydrogen

[1]) Memoires couronnées publ. p. l'Acad. Roy. d. Belgique. **59**. 51 (1899—1900).

ions, so as to render it immediately evident whether the errors attaching to the lakmoïd paper measurement are evenly distributed over that range of the scale for concentration of hydrogen ions here concerned, or not. All the experiments tend to indicate that the error is uniform within the range in question; in other words, that we have here been working within the most favourable field for employment of lakmoïd paper.

The greatest difference found amounts to 0.51 (in Mesembrianthemum echinatum); a so considerable divergence is, however, only apparent in this single instance, although a value of c. 0.4 for this occurs several times. Otherwise, the difference is generally only 0.2—0.1.

	Lakmoïd test	Electrom. measurement	Diff.	Numer. mean.
Mesembrianthemum echinatum	4.9	4.52	+ 0.38	0.40
	4.9	4.65	+ 0.25	
	5.5	5.06	+ 0.44	
	5.6	5.09	+ 0.51	
Mesembrianthemum lingaeforme	4.8	4.61	+ 0.19	0.28
	4.8	4.56	+ 0.24	
	4.9	4.64	+ 0.26	
	4.9	4.55	+ 0.35	
	5.0	4.91	+ 0.09	
	5.1	4.83	+ 0.27	
	5.2	5.02	+ 0.18	
	5.2	4.77	+ 0.43	
	5.3	4.89	+ 0.41	
	5.4	4.99	+ 0.41	
Mesembrianthemum Lehmannii	5.1	4.81	+ 0.29	
	5.7	5.35	+ 0.35	
Kleinia (cuneifolia?)	4.4	4.28	+ 0.12	0.14
	4.8	4.77	+ 0.03	
	4.8	4.68	+ 0.12	
	4.8	4.70	+ 0.10	
	4.9	4.72	+ 0.18	
	5.5	5.29	+ 0.21	
	5.8	5.55	+ 0.25	

	Lakmoïd test	Electrom. measurement	Diff.	Numer. mean.
Diotostemon Hookerii	4.4	4.25	+ 0.15	
	4.4	4.35	+ 0.05	
	4.4	4.35	+ 0.05	
	4.4	4.33	+ 0.07	
	4.9	4.85	+ 0.05	0.09
	5.2	5.13	+ 0.07	
	5.3	5.19	+ 0.11	
	5.4	5.55	÷ 0.15	
	5.4	5.29	+ 0.11	
Cotyledon linguæfolia	4.4	4.23	+ 0.17	
	4.4	4.20	+ 0.20	
	4.4	4.22	+ 0.18	
	4.7	4.53	+ 0.17	
	4.7	4.42	+ 0.28	0.19
	4.7	4.47	+ 0.23	
	4.8	4 63	+ 0.17	
	4.8	4.52	+ 0.28	
	5.5	5.52	÷ 0.02	
Cotyledon coruscans	3.9	4.11	÷ 0.21	
	4.0	4.07	÷ 0.07	
	4.3	4.27	+ 0.03	0.13
	4.5	4.33	+ 0.17	
	5.6	5.43	+ 0.17	
Cotyledon obvallata	4.3	4.04	+ 0.26	
	4.5	4.22	+ 0.28	
	4.6	4.32	+ 0.28	
	4.6	4.47	+ 0.13	
	4.6	4.44	+ 0.16	
	4.6	4.34	+ 0.26	
	4.8	4.35	+ 0.45	
	4.8	4.76	+ 0.04	0.20
	4.9	4.77	+ 0.13	
	4.9	4.74	+ 0.16	
	4.95	4.81	+ 0.14	
	5.1	5.03	+ 0.07	
	5.4	5.46	÷ 0.06	
	5.4	5.06	+ 0.34	

	Lakmoïd test	Electrom. measurement	Diff.	Numer. mean.
Crassula lactea	3.95	4.00	÷ 0.05	
	4.3	4.33	÷ 0.03	
	4.4	4.43	÷ 0.03	
	4.5	4.36	+ 0.14	0.08
	4.8	4.62	+ 0.18	
	5.1	5.15	÷ 0.05	
	5.2	5.13	+ 0.07	
Crassula obovata	4.1	3.96	+ 0.14	
	4.3	4.24	+ 0.06	
	4.6	4.31	+ 0.29	
Rochea falcata	4.05	3.95	+ 0.10	
	4.3	4.14	+ 0.16	
	4.3	4.23	+ 0.07	
	4.4	4.36	+ 0.04	
	4.5	4.29	+ 0.21	
	4.6	4.67	÷ 0.07	0.14
	4.7	4.44	+ 0.26	
	4.8	4.71	+ 0.09	
	4.9	4.76	+ 0.14	
	5.4	5.17	+ 0.23	
	5 5	5.38	+ 0.12	
Aloe arborescens	5.0	4.65	+ 0.35	
	5.0	4.69	+ 0.31	
Aloe cymbæfolia	5.0	4.69	+ 0.31	
	5.2	5.59	÷ 0.39	
Aeonium Haworthii	4.1	4.13	÷ 0.03	
	4.7	4.49	+ 0.21	

Of the 87 cases quoted, only 12 show the error on the acid side; in the remaining 75, the lakmoïd paper throughout shows the concentration of hydrogen ions as too low.

It would seem as if this method of measurement gives errors of unequal magnitude for the different species of plants; thus Mesembrianthemum echinatum for instance, and probably also Mesembrianthem Lehmannii and the two Aloe species, have the errors particularly high, whereas Diotostemon Hookeri and Crassula lactea show only slight deviations. This is of course due to the difference in the substances contained in the sap; we

know from experience with the colorimetric method, that both neutral salts and proteïns, together with the least decomposed products of the latter, can occasion errors in the measurements, and we have doubtless here to deal with similar factors.

It will be seen from the foregoing, that the lakmoid paper test does not furnish any exact means of measuring the concentration of hydrogen ions; it does, however, afford a rapid and convenient method of obtaining an approximate value for the same, and as long as we keep to one and the same species of plant, the error remains more or less constant. The method will be useful more particularly in cases where it is desired to ascertain, without waste of material, whether a plant is or is not at the stage required.

E. OBSERVATIONS ON THE IMPORTANCE OF THE CONCENTRATION OF HYDROGEN IONS FOR THE PHYSIOLOGICAL CONDITIONS IN THE PLANT CELL.

Frequent reference has been made in the foregoing to a point hitherto not fully appreciated in the physiology of plants, to wit, the great importance of the reaction in a substrate for the processes which take place, and for the physico-chemical state of the various substances; it is this feature which will here be discussed in brief.

As pointed out in the Introduction, a complete view of the conditions in a plant can only be obtained when we have made ourselves familiar with the most intricate structure of the individual cells, the more or less distinctly separated sub-divisions of the same, and finally, their content and the processes which take place therein. Mention was also made of the fact, that the methods hitherto known are not so far perfected as to permit of such intensive investigation, for which reason all researches on the subject have up to the present necessarily been confined to rougher analysis.

Only in cases where the sap could be extracted without previous pounding and crushing of the tissue was a clear view obtainable, as for instance with the fluid exuded from the cells in the pitcher of nepenthes, which is entirely analogous with the

juices in stomach and intestine, the blood, or such other liquids as are found in the cavities of animal bodies. We are here certain of the fact that we have to deal with the fluid as actually secreted by the organism.

It is far otherwise, however, in almost every other instance where it is necessary to investigate the sap as found in the cells of the plants, and where crushing of the tissue is necessary in order to extract the material for analysis; we have here no immediate guarantee for the fact that the sap as investigated is actually in the state in which it occurs in the cell; it is possible that we may be analysing a mixture of distinctly different saps from altogether separate compartments. Precisely the same objection may, however, be made in the case of most physiological investigations; from the moment that we have destroyed a cell in order to subject its contents to examination, we can no longer be sure that such contents will remain in the same condition as while still within the normal cell; there is always the possibility that chemical transformation takes place on destruction of the organisation, whereby the normal conditions may be obscured.

From the marked regularity exhibited by the results obtained, however, there would seem no reason to suppose that they are altogether misleading. Moreover, the values found for concentration of hydrogen ions are of such a character that they appear highly probable in the case of cell-saps, as various enzymatic processes are known which would be possible within the limits indicated.

Presuming now, that the results obtained answer to the actual conditions, we are led to the following observations:

In the case of succulents, we note how the concentration of hydrogen ions fluctuates within a certain definite field, these fluctuations, moreover, standing in close relation to the considerable variations observed in the quantity of titratable acid, and to the proportion between such quantity of acid and the amount of malate present. It will thus be a task for future investigations to ascertain how these fluctuations affect the physiological processes.

First of all, it will naturally be interesting to examine how far these variations in the concentration of hydrogen ions affect the actual processes of acid formation and acid decomposition.

As regards the formation of acid, the researches of previous

investigators leave no doubt that this takes place at the expense of the carbohydrates accumulated by assimilation of carbonic acid; the exact course of the process, however, is not known. There is nevertheless every reason to suppose that it is effected through the agency of one or more enzymes. Should it prove that the secretion of acid is an enzymatic process, it will then be interesting to observe how the enzymes in question behave at the different concentrations of hydrogen ions; I hope subsequently to be able to make experimental investigation of this question. The problem can of course only be solved by empirical methods; some remarks, however, may be made upon this head. We may, as a matter of experience alone, take it for granted that a product which remains in a state of solution acts as a check upon the process by which it was formed. We have seen examples of this, for instance in the decomposition processes in the storage receptacles of the nutritive system of germinating seeds, and in the assimilation of carbonic acid in isolated leaves. There is thus every probability that the same rule will apply to the present case; i. e. that the accumulation of titratable acid acts as an impediment to the actual process of acid secretion. Such impediment might, according to Michaëlis' theory already referred to, be presented by increasing concentration of hydrogen ions, as reducing the activity of the enzymes concerned by placing them under conditions unfavourable to dissociation; i. e. if the non-dissociated portion of the substances possessing the enzymatic qualities be the active part, by an increase in the degree of dissociation of the substance in question; or, if a dissociated part be the decisive element, by retarding the dissociation. If this hypothesis prove well-founded, then we have here an excellent instance of the power of a cell to regulate the processes of metabolism; a quality which is known from numerous processes, albeit its precise nature has, in the great majority of cases, not yet been elucidated in detail.

Next, as regards the process of acid decomposition, it is also important to know how far this is dependent upon the concentration of hydrogen ions. From the investigations of Spoehr, mentioned in the historical section, it appears that the action of light has a marked decomposing effect upon the acids in question, while the researches of de Vries and others again have shown that the titratable acid may even in darkness altogether dis-

appear, if the plant be left long enough. It would thus also seem not unlikely that a constant decomposition of acid takes place quite apart from the action of light, this being, however, concealed when the acid secretion is equally strong or stronger. The process of acid decomposition also is doubtless dependent upon the reaction of the substrate, and an investigation of this would thus in all probability contribute to the elucidation of all regulatory processes which take place in the cells.

Besides the actual formation or decomposition of acid, all the remaining processes which take place in plant cells would require to be investigated in their relation to the factor in question. Such investigations would, at least in part, doubtless present serious difficulties, at any rate as regards processes other than those effected by enzymes which may be isolated from the cell. As regards the synthetic processes, these have up to now been investigated only to a very slight extent, and it is hardly likely that any great progress can be made for some time to come.

On the other hand, such investigations would furnish information as to how far the results obtained with regard to concentration of hydrogen ions correspond to the actual conditions in the living cell; if the range for concentration of hydrogen ions within which the enzymes occurring in the cell can carry out their function be found to coincide with those noted in the extracted sap, we should then have circumstantial proof of the validity of the methods employed. Even if some few of the enzymes present should prove to be active only outside the section of the scale for concentration of hydrogen ions here concerned, the results arrived at might still be of value, as there is nothing to prevent the occurrence of such enzymes in cavities containing liquid which in volume makes up only an inessential part of the main quantity of all sap, and having a concentration of hydrogen ions differing slightly from that found.

Finally, there remain the chemico-physical conditions in the cell. The state in which the plasmatic membrane may occur, for instance; might not this be supposed to be affected by alterations in the concentration of hydrogen ions? Is there not a certain likelikood that the substances of which it is composed may change their character when the reaction of the cell-sap is altered? But again, if this is the case, could it occasion alteration in the

translocation whereby substances previously isolated were brought into contact one with another?

Also the osmotic conditions in the cell however, are probably subject to change on alteration of the concentration of hydrogen ions; not only owing to the fact that a transformation of carbohydrates into acid occasions an increase of the osmotic pressure, but also by the fact that the increase in concentration of hydrogen ions involved by an increase in the quantity of acid gives rise to an alteration in the degree of dissociation of the other substances present in the cells, which can thus contribute to the fluctuations in the osmotic pressure, or possibly prevent their occurrence, — acting, in fact, as a "buffer" in osmotic respects.

Obviously, the elucidation of these and many other questions will be of great interest, and it is possible that those succulents which exhibit the greatest fluctuations in concentration of hydrogen ions may furnish the clearest solution.

Hitherto, only succulents have been mentioned, as these formed the main object of the present investigations: as a matter of fact, however, practically all that has been said in this chapter with regard to these plants may be applied to non-succulent plant organs, since these do not differ essentially, but only in degree, from the former.

SUMMARY.

A. The following are the results arrived at with regard to the sap from leaves of succulent plants:

1. The concentration of hydrogen ions in the sap varies from $10^{\div 3.9}$ to $10^{\div 5.7}$.
2. A certain relation has been found to exist between the concentration of hydrogen ions and the quantity of acid titratable to litmus point.
3. The concentration of hydrogen ions is determined by the quantity of titratable acid and the quantities of dissociated malates.
4. In the sap of Rochea falcata, Cotyledon obvallata and Cotyledon linguæfolia (possibly in succulents generally) acid is

never found together with acid salt; varying quantities of acid salt and normal salt do, however, occur, and this mixture is of a marked "buffer" character.

5. The faculty of forming and accumulating acid varies greatly in the different species of succulents; it is not proportional to the degree of "fleshiness" of the leaves, but there is a certain likelihood that it stands in causal relation to the quantity of dissociated malates. which again will depend upon the quantity and kind of the bases of which these are composed.
6. The sap shows, on titration, a considerable distance between the litmus and phenolphtaleïn points, the different species varying greatly in this respect.
7. In certain species (such as Diotostemon Hookeri and Crassula lactea) this difference was found to be greater in old leaves than in the younger.
8. The quantity of the substances responsible for this difference is, at any rate in some degree, dependent upon the aluminium malate present in the sap.
9. In the case of Rochea falcata, substances of highly inconstant character were found, appearing as acids at the lower concentrations of hydrogen ions ($10 \div 7$ or possibly already at a more acid stage). These substances are easily oxidised, and then assume the character of stronger acids. Similar substances, or possibly the same, occur in all the succulents investigated, but judging from the results of titration, in greatly varying quantity.

B. In seedlings of Lupinus albus, the sap extracted from the axis showed very nearly uniform values for concentration of hydrogen ions, the limits found being $10 \div 5.78$ and $10 \div 6.03$, even where considerable variation is apparent in the quantity of acid titratable to litmus point.

C. In the fluid from non-stimulated pitchers of Nepenthes the concentration of hydrogen ions appears to be altogether indefinite, whereas in the case of a pitcher subjected to stimulus, it would seem to be constant, and greater than $10 \div 7$.

D. By means of lakmoïd paper, an approximate value is obtainable for the concentration of hydrogen ions in a liquid when such concentration falls within the limits of $10 \div 3.8$ and $10 \div 6.0$.

NOTE.

In the foregoing I stated, that as far as I knew, no measurements of hydrogen ions had previously been made in the juices of plants. A few months back, however, there appeared a work by Wagner[1]), on the alteration in concentration of hydrogen ions in the cell-sap of plants under pathological conditions brought about by the action of bacteria. Wagner used for his experiments:

Sinapis alba	the cell-sap of which generally exhibits values for p_H of:	5.5
Brassica oleifera		5.65
Solanum tuberosum		5.8
Sempervivum Hausmannii		5.4

Of these, the three first lie close to the values found by me for non-succulent plants (Lupinus and barley).

In all plants, it was found that the concentration of hydrogen ions after the inoculation with bacteria first fell a little (generally c. 0.05 in p_H), thereafter rising more or less as the bacteria gained the upper hand and destroyed the plant, or the plant succeeded in withstanding the attack. The highest value found by Wagner for concentration of hydrogen ions was $10^{\div 4.7}$ for Sempervivum. He makes no mention of the fact that this plant should under normal conditions also exhibit variation in the concentration of hydrogen ions in the cell-sap; a variation probably due to its thick, fleshy leaves.

In measuring the concentration of hydrogen ions, Wagner employed a micro-colorimetric method; a variant upon the colorimetric method described by S. P. L. Sörensen. The indicator used was lakmosol, a constituent of lakmoïd, dissolved in ether, with the addition of a small quantity of collodium. This method is, he states, practicable within limits of $10^{\div 4.2}$ and $10^{\div 6}$, which are about the same as those noted by me, $10^{\div 3.8}$ and $10^{\div 6}$.

[1]) Zeits. f. Bakter. u. Parasitenk. II **44**. 708—719 (1916).

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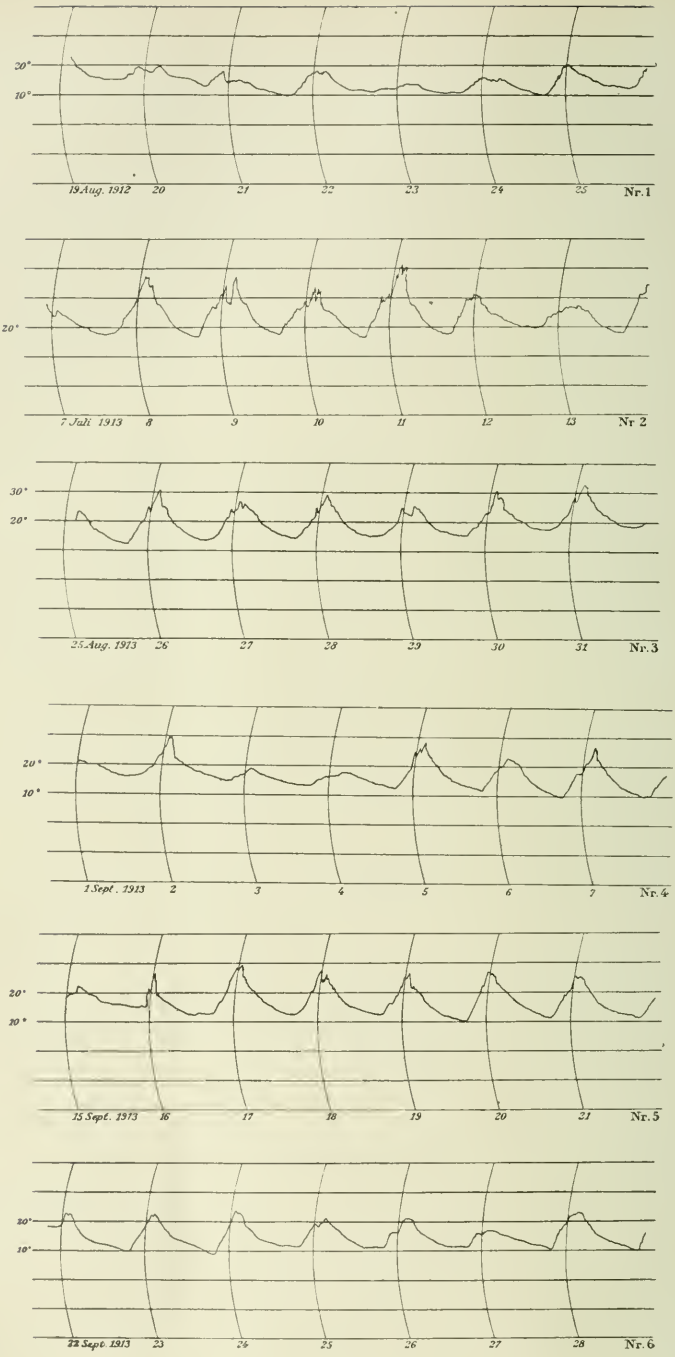


Temperature-curve.

THE CHROMOSOMES.

THEIR NUMBERS AND GENERAL IMPORTANCE.

BY

Ö. WINGE.

With 1 Plate and 46 Figures in Text.

> "L'un des faits les plus importants, mis en évidence chez les plantes, consiste dans la fixité du nombre des éléments chromatiques des noyaux sexuels."
>
> *L. Guignard*, 1891.

THE greatest morphological resemblance between plants and animals is that found within the walls of the cell, and more especially in the qualities of the nucleus. In the cell — and here alone — do we encounter elements which are in the strictest sense of the word homologous for the two great groups of living matter. And the occurrence of the chromosomes: the small colourable bodies which continually make their appearance during the process of nuclear division, is in a remarkable degree common to most plants and animals.

An element of so ubiquitous a character, that it may almost be said to be characteristic of living matter generally, must naturally be presumed to be of fundamental importance for the existence of the living organism. And we find also, that the chromosomes have gradually come to be regarded as of quite extraordinary importance, the more so since the technical improvements in microscopy have rendered it possible to penetrate further and further into their nature.

Students of heredity also, have shown considerable interest in these bodies, the peculiar behaviour of which during reduction division, fertilization, etc. — in a word, during sexual propagation — offers them, as it were, tangible evidence as to the correctness of the principles experimentally arrived at. On the other hand, however, it must be noted that certain authorities on heredity themselves oppose the tendency to allow the chromosomes a too direct importance in this respect: i. e. as the seat of genotypic disposition.

The fact that each species of plant or animal has as a rule a certain and constant number of chromosomes in the nuclei, more

particularly those of the sexual cells, has, from the very commencement of chromosome research in the 70's, incited cytologists to ascertain, if possible, the precise number of chromosomes for each separate species of plant and animal. The minuteness of the chromosomes themselves, their variability and frequently inconvenient position, render the work far from easy, but in the course of years one investigator after another has continually furnished contributions, with the result that we know now the numerical value for the chromosomes in several hundred species of both kingdoms.

As long as the nature of the chromosomes is still comparatively little understood, comprehensive investigations as to their character and occurrence generally will necessarily be a matter of difficulty; one line of approach, however, yet seems to be clear: to wit, by the study of this very feature: the number in which they are found in the various organisms. In epitomizing what is known as to chromosomes generally, it would be natural to commence with the simplest factor, i. e. their numerical value. If we can then succeed in discovering any laws or principles governing the numbers characteristic of each organism, such knowledge will in itself afford a better foundation for further study.

In seeking to form some preliminary idea as to what features in plants might be imagined to determine the numerical value of the chromosomes, an embarrassing number of possibilities suggest themselves. The value in question might for instance be supposed to stand in close relation to the phylogeny of the particular plant — or to the volume of the cells, the size of the plant itself, or possibly to the numerical proportions in the external organs, or physiological conditions, or the number of genes etc. There is thus no lack of plausible hypotheses; only by critical investigation of the numerical values actually found for the chromosomes can we determine which theory is — or which theories are — correct, or perhaps, per exclusionem, which are not.

When I commenced this work, in 1914, my intention was to collect, as far as possible, from the literature already extant, all the known values for number of chromosomes in the vegetable kingdom, in order thus to form some view as to the importance of their numerical value. No such work had up to that time appeared, and there seemed good reason to believe that one might, by statistical methods, arrive at results which the invest-

igation of single plants had not sufficed to afford. Here, however, apart from the ordinary difficulties inseparable from such compilatory work, such as that of procuring, and reading through within a reasonable time, the necessary publications, I encountered further hindrance in the fact that the various authorities consulted frequently disagreed. There are numerous plants whose number of chromosomes is still not known with certainty, despite considerable research, the different results arrived at by several investigators in each case leaving us in doubt as to which of them is correct. Statistics of this nature would soon become worthless if incorrect statements were allowed to creep in, and even though the bulk of them were indubitably correct, it would obviously be impossible to rely completely upon conclusions drawn from such material. I have therefore restricted myself to the procuring of information as to the numerical value for chromosomes in a considerable number of plants, and have more particularly endeavoured, by personal investigation, to elucidate the importance of the chromosomes, and of their numerical value, especially within a certain class of related forms. A further reason for thus departing from my original plan was the fact that I had already, from the statistical results obtained through my study of the literature, secured confirmation of the most important points which I had expected to arrive at by a thorough statistical investigation.

As is frequently the case when one is occupied with a task of any general interest, a kindred work appeared while I was thus engaged, to wit, Tischler's excellent treatise entitled "Chromosomenzahl, -Form und -Individualität im Pflanzenreiche" (Progr. Rei Botanicae, Vol. 5, 1915) which deals with the question of chromosome numbers in plants more thoroughly than has hitherto been done. Tischler's work, is, according to its purpose, more particularly a survey of our present knowledge on the subject, and the writer does not therefore go so closely into the theory of chromosomes and their numerical value as is necessary for a thorough comprehension of the question. My present work, therefore, which is especially directed towards the theoretical aspect of these chromosome numbers, will, I trust, hardly be superfluous.

The science of cytology has great tasks before it, and not least important is the function it has acquired as a most neces-

sary discipline for various branches of hereditary research. Nevertheless, the chromosomes and their importance, — central features, so to speak, in cytology itself — are still frequently disregarded in natural history research, though this is mostly found to be the case among non-cytologists.

It is still, of course, a question, how great importance we should attach to these peculiar elements; yet I venture to assert that if the chromosomes and their behaviour were easier to observe, and thus more generally known, then those who believed in their greater significance — which need not here be more precisely defined — would form a large majority.

The present cytological investigations were for the most part carried out in the Physiological Department of the Carlsberg Laboratory, to the Head of which department, Dr. Johs. Schmidt, I am indebted for the facilities accorded me in carrying out the work. I also wish to thank my very respected tutor, Prof., Dr. Eug. Warming, who has throughout taken an interest in my researches, and Dr. Ove Paulsen, Amanuensis at the Botanical Gardens in Copenhagen, who kindly undertook the fixation of some plant material at a time when I was prevented by absence from doing so myself. Finally, I beg to express my thanks to the Trustees of the Carlsberg Fund, who in 1911 furnished me with funds for studies at the universities of Stockholm, Paris and Chicago, and i 1914-1915 with the means for carrying out the present work.

CHAPTER I.

GENERAL OBSERVATIONS ON THE ALTERNATION OF GENERATIONS AND NUCLEAR MORPHOLOGY.

The great majority both of plants and animals traverse continually the cycle known as the alternation of generations. In the higher forms of both kingdoms, we find that each individual comes into existence, not directly through division of its predecessor, or as an offshoot of the same, but through the intermediate stage of a new generation, consisting as a rule of units quite different in appearance, which by partial or entire fusion again bring forth the next replica of the original. In this continual cycle, the most generally known stage of the process is that of fertilization; i. e. the union of a male with a female element, save for which the highest organisms at least of our earth would be doomed to extinction.

In this cycle of generation, vegetative propagation of what kind soever is of entirely subordinate importance, and forms, as it were, but a series of bypaths ever leading back to the main road. And in this connection, the question of chromosomes is of the highest importance for a proper comprehension of the whole problem; only by the study of these elements can we attain to any clear conception of the entire process.

The earliest conjectures of science in this field supposed the existence of a "substance" by which fertilization was effected. The first step in the right direction was the discovery of small living and locomotive organisms, the sexual cells, in *Fossombronia* and others, about the middle of the 18th century. Seen under the microscope, these "Spiralfaden" exhibited a marked resemblance to the independently living flagellata, and were also regarded as genetically independent of the productive power of the plants themselves.

Nearly a century later it was found that the spermatozoids were really formed by the plants themselves; following on this

discovery, corresponding cells were gradually recognized in all kinds of animal and plant forms. Hofmeister (1851) is the true discoverer of the alternation of generations in plants. In the course of the next decade or so, the rule was formulated that two sexual cells, a male and a female, fused together, forming a germ, which by division developed into the wonderfully complex arrangement of cells of which the higher organisms is composed. Animals and plants again gave off unicellular sexual products, and so on — the alternation of generations was now to a certain extent made clear.

The study of chromosomes, however, increased the value of this discovery to an enormous extent. The researches of the seventies and eighties gave us, with the results arrived at by van Beneden, Strasburger, Guignard, Boveri and others, the first knowledge as to the constancy of the number of chromosomes in species, and it was shown that the sexual cells continually exhibited a number only half that of the remaining cells in the individual, and that this halving of the number of chromosomes took place according to a certain definite plan, not found elsewhere in the ontogenesis of living things. Weissmann called this cell division, whereby the number of chromosomes present was reduced to half, "reduction division", the term itself indicating the nature of the peculiarity which it referred to.

A new side of the alternation of generation was thus thrown open. The most generally known organisms in the animal and vegetable kingdoms had all one thing in common: they all formed sexual cells having half the original number of chromosomes, and the fusion of two such cells on fertilization gave once more an organism with the full number of chromosomes. The one generation, having a number of chromosomes a, was now termed the sporophyte, or non-sexual generation, the second, with $^a/_2$ chromosomes, being designated the gametophyte or sexed generation. The two generations alternated one with the other, the sporophytes by reduction forming gametophytes, and these again by fusion, called fertilization, sporophytes.

We have in the foregoing taken the sporophyte as starting point, this being usually the better known and more highly developed form, and that described by systematists. As a matter of fact, the original form is beyond question the gametophyte, the sporophyte being, from the phylogenetic point of view, a

later modification, derived from the fusion of two original individuals. The sexual cells, or gones, (Lotsy 1904) are homologous with the non-sexed ancestors of the existing species, "systematic species", and exhibit in their structure, even among the most highly developed organisms, a remarkable resemblance to the simplest unicellular independent organisms now inhabiting nature; the protozoa. In the course of evolution, the two originally unicellular organisms have, by fusion in pairs, acquired the power of developing into greater cell-complexes, with a marked division of function. Taking the gametophyte as the original, we designate its number of chromosomes by x, that of the sporophyte by 2x — or call them the haplophyte and diplophyte respectively.

The greatest resemblance between plants and animals is, as already mentioned, the alternation of generations with its peculiar cytological processes. In some forms, the sporophyte is developed, by ordinary vegetative cell division, to a high degree of perfection, while the ontogenesis of the gametophyte is extremely brief and simple; in other organisms the gametophyte will rank with the alternating generation. The scheme according to which animals and plants propagate is in all essentials the same.

The simplest alternation of generations is that found in the vegetable kingdom in organisms such as the unicellular green algæ.

The conjugates *Cylindrocystis* and *Closterium* will serve to illustrate a simple alternation of this sort, albeit the chromosomatic conditions are here not altogether elucidated. The forms in question are unicellular organisms (gametophytes), which by fusion in pairs form zygotes (sporophytes), these again dividing up by reduction division into four daughter cells (gametophytes). The cycle is here restricted to three generations of cells (Fig. 1). True it should be added, that the gametophyte further possesses the power of dividing itself by simple vegetative transverse division; this fact, however, does not affect the actual alternation of generations, though it may be of great importance for the existence of the organism. — In certain forms belonging to this category, we find that after reduction, not all the gametophytes come to developement; two or three of them may perish, and in such case, the vegetative division of the gametophyte must of course be the

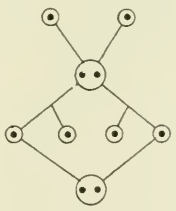


Fig. 1. Schematic view of the simplest alternation of generations.

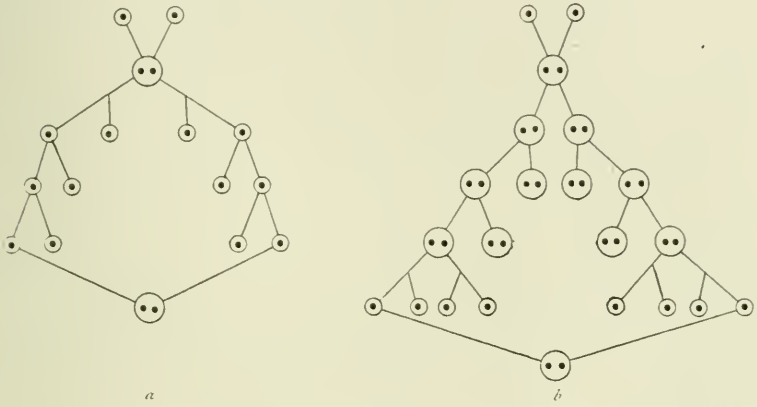
true origin of propagation, as each fertilization involves a loss of individuals, or at best, when only two are lost, (*Closterium*) serves to maintain the number.

An organism among the *Chlorophyceæ* such as the *Volvocacea Eudorina elegans* exhibits a corresponding simple alternation of generations. This unicellular alga, which lives in colonies, has two kinds of individuals (gametophytes) differing in size and appearance. Two cells of different appearance, answering to male and female, copulate and form a zygote. Though we already here, in unicellular forms, encounter a morphologically distinct sexual difference in the individuals, yet here, as in the conjugates above mentioned, each individual as a matter of fact represents an egg, or a spermatozoid respectively (Boveri 1902), the haploid generation consisting exclusively of such; and these form, by total fusion, a diploid zygote, which, without further differentiation, forms gametophytes with half the number of chromosomes.

These examples in themselves at once invite us to consider why this periodical fusion and separation of individuals takes place: what can be the importance of the alternation of generations to the species; or if the whole thing is but a freak of nature. Before taking up this subject for discussion, I will first briefly call to mind how homogeneously the alternation of generations and consequent doubling or halving of the number of chromosomes takes place throughout the two great divisions of living matter, the animal and the vegetable kingdom.

With this end in view, I will offer yet one more instance of the alternation of generations, taken from among the lowest organisms, to wit, the parasitic *Plasmodiophoraceæ*, whose systematic position according to their whole biology lies on the boundary between plant and animal life; and specially related to both *Chytridiaceæ* and protists (trypanosomes). The course of the alternation, as shown for these organisms (Maire et Tison 1909, Winge 1912, Kunkel 1915 and others) is as follows: unwalled myxoplasms (sporophytes) with few nuclei, are found in the cells of the host plants. Cell division and plasmatic division take place, whereby a great number of multinuclear myxoplasms are produced. Reduction sets in, and haploid, unicellular spores are formed by the building up of a wall about unicellular plasma parts. The germination of the spores liberates swarming-spores,

the further fate of which is still but little known, but which probably copulate in pairs (nuclear fusion must take place somewhere in the cycle, in order that the number of chromosomes may be maintained) whereby the diploid generation again arises, and in the form of myxoplasms penetrates the cell walls of the host plant, there to undergo vegetative division once more. Even though the point of time at which fertilization takes place is thus not definitely determined, it is nevertheless clear that the cycle in these organisms differs from that in those before men-



a *b*

Fig. 2. Schematic view of the alternation of generations in *Conjugatæ* (*a*) and *Plasmodiophoraceæ* (*b*).

tioned; we have here a series of vegetative divisions intruding as an obligatory phase in the ontogenesis of the sporophyte, whereas in those previously referred to, the vegetative propagation was allotted to the gametophyte. (See Fig. 2).

We have now mentioned some forms, in which the alternation of generations is extremely simple, and among these, on the one hand those where the vegetative division falls to the haplophase, and on the other, those where the diplophase includes the vegetative division.

By selecting further types from the botanical and the zoological world, we might show how the more highly specialized organisms have a greater vegetative reproduction period thrust in between the fertilization stage and the reduction of the zygote: i. e. in the sporophyte. The difference between the mentioned unicellular forms and the more complicated plants and animals in this respect is in reality not very great. If the daughter cells

formed by the intermediate vegetative division of the sporophyte in these unicellular organisms were not separated one from another, but remained united throughout several generations of cells, we should after only a few generations have a considerable complex of cells; i. e. a multicellular, sporophytic organism. Hertwig in his "Allgemeine Biologie" sufficiently emphasized the importance of regarding the cell as unit. Multicellular organisms may perfectly well be considered as colonies of unicellular ones. The fact that more highly developed animals and plants are not generally regarded as colonies, or as homogeneous cell-masses, is due entirely to the division of function, the cellular differentiation — organ formation — which takes place in a continually increasing degree as we near the top of the most highly developed branches on the genealogic tree in either kingdom.

The higher the developement of the organisms we consider in the vegetable world, the more we find the gametophyte reduced in comparison with the sporophyte. It will suffice to mention that even in the mosses, and more particularly in the liverworts, the gametophyte is an independent plant, whereas the sporophyte is less conspicuous, and so dependent upon the gametophyte on which it is formed, that it never attains to any independent existence. In the pteridophytes, the sporophyte is the more conspicuous of the two, the gametophyte being highly reduced, especially in the heterosporous forms. In the gymnosperms, the sporophyte is enormously developed, and nourishes the gametophyte, which is entirely enclosed in its tissue. As shown by Chamberlain (1905) we may further, in *Gnetum*, *Sparganium*, *Peperomia* etc. observe a transition to still more reduced gametophytic organisms, the number of cell divisions decreasing, and wall formation being partly omitted, until at last we have only nuclear division: i. e. multinucleate gametophytes. In *Lilium* and several other angiosperms, the female gametophyte thus consists of only 8 cells, or more correctly of a cell with eight nuclei, produced by three successive nuclear divisions. Since 1905, still simpler types have been found, and a short time ago, Dahlgren (1915) crowned the work by pointing out, in *Plumbagella*, a type where the female gametophyte merely developes to a cell with four nuclei, one of which becomes the egg nucleus.

And now, in the animals, we find the female gametophyte reduced in precisely similar degree, to 4 cells. Two unicellular

polar bodies are thrown off from the ovocyte, one of which divides up into two, and the remaining fourth cell represents the egg. The homology is beyond doubt. Fig. 3 shows the egg formation in the most reduced gametophytes of both phanerogams and animals

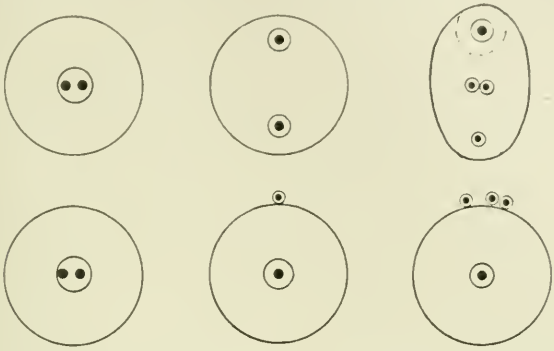


Fig. 3. Schematic view of oogenesis in higher plants (uppermost) and animals (below).

Passing now to consideration of the male gametophyte, we find that this has, in more highly developed forms, undergone an exactly similar reduction, and in order to avoid repetition, we may dispense with further discussion of these. In both animals

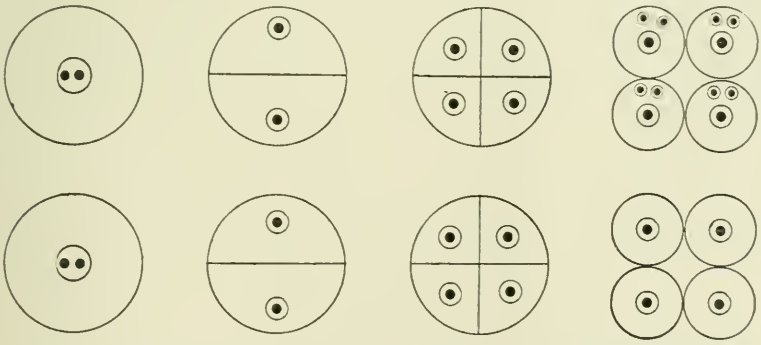


Fig. 4. Schematic view of spermatogenesis in higher plants (uppermost) and animals (below).

and plants the developement proceeds in entirely the same way; save that the nucleus of the gamete will, as we know, in plants divide in such a manner that as a rule 3 or even more nuclei (5 in *Scilla* and *Ornithogalum*; Strasburger 1884) may be formed by the germination and growth of the pollen grain, while the animal gametophyte is always mononucleate. Fig. 4 shows the full ontogenesis of the male gametophyte in animals and plants.

From the simplest unicellular organisms having independent life and propagating vegetatively with x chromosomes, — and only occasionally copulating in pairs to form a unicellular 2x generation — we have first passed on a short step to forms where the 2x generation divides, not to revert to the haploid phase until after the lapse of several cell generations. And finally, we arrive, albeit after but superficial consideration, at the highest animal and plant types now existing, in which the gametophyte has dwindled to an altogether ephemeral and dependent organism, without the power of vegetative reproduction. We then note the peculiar fact that many of the lowest plant organisms, which have been given their name by systematists, are gametophytic forms, whereas in the most highly developed plants, the specific idea and description of the species are seen to be based throughout on the sporophyte. This again calls to mind the fact that the sexual cells — eggs and spermatozoids — of more highly developed plants (and animals) are homologous with the haploid ancestors of these organisms, whereas the sporophyte is a remarkable double existence — or rather a colony of such.

That the periodical reversion to the original form — however brief it may be — is of great importance for plants and animals will doubtless be generally admitted, and we may in the following, presupposing the gametophyte as the original organism, consider what advantages may be thought to accrue to the living form thereby.

In seeking for any such "advantage" in connection with the principle of alternation of generations, we need not imply any inclination towards the idea that nature generally tends in the direction of expediency. Such teleological explanation lacks all justification, the more so, since nature often works in opposition to the interests of her own products (parasitism, for instance). It is nevertheless beyond doubt that the best equipped individuals, species or biotypes — it is here immaterial which theory of evolution we hold — are better qualified to make a good career and enrich nature with their progeny[1]) than are others less fa-

[1]) That individuals best equipped in other respects may owing to inferior fertility be excluded from this is of course reasonable enough. It will then depend on circumstances, whether the advantageous qualities possessed can give the organism sufficient power of resistence to secure its continued existence despite its lack of fertility. The "cultured" races of mankind may fairly be taken as example.

voured; in other words, that nature exerts a natural selection. If then we now, long after the status nascendi, seek to reconstruct the ways by which development has proceeded, we have every right to look for advantageous qualifications which can be supposed to have given the organism possessed of them a better chance in the struggle for life.

Presuming then, that unicellular organisms even in the past at times propagated sexually, much as such organisms now do, we have to consider what advantages even a brief fusion of two individuals could have had for their further existence.

As the division of cells is inseparable from all continued vital activity, so also the distribution of the contents of the mother cell among the daughter cells is of decisive importance for the possibility of existence in the organisms. The course of cellular division is naturally affected by surrounding conditions. Any unfavourable physical or chemical influence may give rise to irregularities in the division process, this again producing deformed cell individuals, or at any rate individuals differing in value from those whence they proceed. Nature produces many abortions which are doomed to perish; many such are known to us through theratology, and numbers of others die off at so early a stage of developement as to escape our observation. Such deformities, which are best known among the higher plants, but which of course may equally well be found in unicellular organisms, necessarily indicate that the laws by which living nature proceeds may fail at times — particularly, no doubt, owing to varying influence of different external conditions, and it has been found possible, as we know, to produce by experimental means certain cyto-theratological phenomena as the result of arbitrary external influence (e. g. Němec 1910). We may therefore take it for granted that unicellular organisms will, in the course of manifold vegetative reproductions, have produced a progeny not altogether homogeneous. Various vegetative individuals of the clone in question will have arisen, differently adapted to their surroundings, some having, in process of cellular division, lost one element, others another of those present in the original mother cell, and making for more perfect suitability to prevailing conditions. It is now reasonable to suppose that two individuals on fusing together would to a certain degree be able to replace what might be lacking in each; it seems at any rate likely that

the zygote, as a product of the two individuals, may be regarded as better adapted to its surroundings than either of the two whence it was derived. After fertilization, separation takes place, and we then presume that both individuals would as a rule have derived some benefit from the temporary union, inasmuch as they will have been able in some degree to help out each other's shortcomings. We need not here go further into the discussion as to mutual influence of gametes during fertilization; the cytological phenomena as visible under the microscope, and the question of their theoretical importance, must be left to a later chapter; we shall then also refer to the conditions which appear to support the theory advanced.

A continually repeated intermingling of individuals during the diplophase will afford the greatest likelihood that the species may retain the substantial elements which determine the entire nature of the organism. An individual failing in a (gametophytic) individual may be repaired in its subsequent fusion with another, so that the gametophytes again formed by the double organism leave the diplophase in better condition than prior to fertilization. The essential importance of fertilization would thus be, that it enables individuals, especially the gametophytes, to supplement each other.

This idea is to a certain extent related to that advanced by Weissmann (1892) according to which, fertilization and the alternation of generations essentially result in a "Vermischung der Vererbungstendenzen zweier Individuen". Weissmann, however, attached chief importance to the variation of the progeny in appearance and structure, which he supposed to be greater after such "Vermischung", this then again giving selection a larger and better material to work on.

Albeit the higher plant and animal individuals really are of a double nature, they nevertheless appear and act as harmonious units, and the dualism is, from a morphological point of view, rarely observed. On fertilization, egg and seed cells are as a rule entirely fused together, and the male and female chromosomes distribute themselves in an apparently uniform manner through the nucleus of the zygote. We find, however, instances where a relative independence may make itself felt even after fertilization. I disregard her to some extent the peculiar synkaryon of the *Uredineæ*, where fusion of two cells takes place prior to

the formation of the æcidiospores, but without fusion of the nuclei. The æcidiospores and the hyphæ thence derived, which later form uredospores and teleutospores, remain binuclear, and not until the teleutospore stage do the nuclei unite. If then we allow the nuclear fusion to be decisive for the moment of fertilization, the *Uredineæ* afford no example of dualism, and the same applies to certain entirely corresponding conditions found elsewhere, as for instance in some conjugates where the two original nuclei of the zygote remain separated until the germination of the zygote itself, or again in the animal kingdom, with the small snail *Crepidula*, where the two nuclei are separate throughout most of the animal's life.

Also after fertilization, however, i. e. after the union of male and female nuclei, we may find the parent chromosomes remaining separated (gonomery), or even after the embryonal division have commenced. In *Juniperus* (Norén 1907, Nichols 1910) and *Tetraclinis* (Saxton 1913) for instance, we find that the parent chromosomes, after completion of fertilization — i. e. after the sexual nuclei are entirely fused — again separate, each to form its own spindle during the first embryonal division. Ferguson (1904) has even found, in *Pinus*, the parent chromosomes separated during the second division in the proembryo. These important observations show that fertilization does not consist of a complete intermingling of the elements in the gametal nuclei, but that these on the contrary, continue to be individualized to no slight degree. Even more distinctly, if possible, is this shown by examples from zoology; Rückert (1895) and Häcker (1895) have, in cytological investigation of the fertilization in *Cyclops*, found the parent chromosomes individualized even through several generations of cells, while Moenkhaus (1904) has noted the same thing in the hybrid form *Fundulus* × *Menidia*.

It is thus likewise proved, that fertilization only occasions a collecting of the chromosomes from nuclei of the gametes in the fusional nucleus of the zygote, the chromosomes themselves not fusing. The same is further shown by other investigations; in *Pinus*, Blackman (1898) and Chamberlain (1899) and Ferguson (1904) have seen the chromosomes of each of the sexual nuclei forming even before these nuclei had fused, i. e. before fertilization, and the chromosomes thus remain separate during the prophase of the first embryonal division (spindle formation).

Similar conditions have been noted in *Larix* (Woycicki; see Coulter and Chamberlain 1910), *Tsuga* (Murrill 1900) and *Cunninghamia* (Miyake 1911); probably also several others behave in like manner.

Altogether, it is extremely doubtful whether chromosomes are able to fuse in pairs, so as to reduce the number of chromosomes to one-half. Normally, this will hardly take place. The opinion of Fraser and Brook (1909) tending in this direction must be explained as due to the fact that their investigations were made with *Ascomycetæ* such as *Humaria granulata*, *Ascobolus furfuraceus*, *Peziza vesiculosa* and *Otidea aurantia*, where they believed to have found, like Harper (1905) in *Phyllactinia*, two fertilizations in the cycle of these fungi. The assumption of two successive fertilizations, and subsequent appearance of 4x chromosomes in the zygote, necessarily obliges the investigator to suppose two reduction divisions, or a halving of the number of chromosomes either by fusion of the chromosomes in pairs, or by other means. According to Fraser and Brook, the chromosome fusion should take place in the young ascus after the first heterotypic division. In the same way, Harper is obliged to have recourse to a similar theory; he believes to have found 8 chromosomes both in the primary *Phyllactinia* gametophyte and in the ascogenous hyphæ. We may altogether disregard these remarkable and still greatly disputed cases, which are, moreover, without essential importance to the main question.

The previously mentioned instances of cytological conditions in the fertilizing nuclei remind us once more of the fact that the germ formed by fertilization is a dual existence, produced by the addition of two originally separate organisms — and that each sporophyte, despite the picture of unity and harmony which it presents, has the same dual character.

Unsuccessful attempts have been made to compare the cycle of the chromosomes during alternation of generations with that of various other morphological elements. Zoologists have endeavoured to make such comparison, for instance, between the constancy of the number of chromosomes and that of the fingers on a human hand. Child (1910) observes in this connection (p. 118): "Physiologically it is no more difficult to conceive that a piece of nucleus should under certain conditions give rise to the characteristic number of chromosomes, than that a piece of

the actinian body should give rise to the characteristic number of tentacles". And Fick (1906) likewise compares the chromosomes with various elements of the body, such as teeth, feathers, fingers, which similarly occur in a certain number and form for each distinct species.

Such parallels are in my opinion unjustifiable. In the first place, no other element within the walls of the cell can be compared with the chromosomes. True, some investigators have found that the chromatophore in *Anthoceros*, for instance, divides during the vegetative reproduction previous to each cell division (Schimper, Meyer, Scherrer 1914); that the chromatophores are not, as Lewitsky, Pensa, Guillermond have found in certain other plants, derived from chondriosomes; but in formation of the sexual cells, no chromatophore was found in the spermatozoid mother cells, so that the embryo on fertilization only received that proceeding from the mother (the egg cell) — and even if other elements in the cytoplasm have the power of dividing, and even of reaching the embryo cell with both sexual cells, there is nevertheless in none of these anything approaching the regular system of division which distinguishes the chromosomes. And in the second place, is must naturally be altogether unwarrantable to compare the constancy of chromosomes with the occurrence of a fixed number of limbs, etc. in species. Members, segments, stamens, feathers etc. are as matter of fact not transmitted at all as such, but merely as morphologically non-differentiated factors inherent in the sexual cells, whereas the chromosomes are literally inherited, by direct transmission, from cell to cell; from generation to generation. Nothing in living nature is more directly hereditary than these very chromosomes, and I can only regard it as incomprehensible, that anyone should attempt to pass off the numerical constancy of such elements with a reference to the fact that other elements of a totally different nature also are constant.

With regard to the sporophyte, the constancy of the number of chromosomes in species is here somewhat restricted. It is extremely common to find, in sporophytic tissue which can have no direct share in the propagation of the species, a number of chromosomes deviating from the normal. Numerous cell individuals, whose life is only brief, or having a special mission, such

as for instance the transport of nourishment, have frequently not 2x chromosomes in the nucleus, but either fewer or more.

As I believe to have shown on a previous occasion (Winge 1914) the nuclei in plant cells which are obliged from the nature of their function in the individual to afford passage for considerable quantities of matter often have far more than 2x chromosomes, this being arrived at by mitotic divisions of the cells in question, whereby they become binucleate or multinucleate, when the daughter nuclei fuse together, and form a didiploid or pluridiploid nucleus. Such cells may often, however, remain binucleate or multinucleate, no fusion of daughter nuclei taking place; in such cases, only the cell will be hyperchromatic, not each individual nucleus. This point we shall have occasion to refer to in a later chapter.

The sporophyte may also, as we know, often be found to contain a number of chromosomes less than 2x. The extant literature affords so many instances of this comprising variations of the same thing, that I may here regard it as sufficient merely to note the fact. The ovarial tissue in flowering plants may doubtless be taken as affording particularly frequent testimony as to this.

Gametophytic nuclei may also at times be hyperchromatic, as is often the case for instance with the vegetative nuclei of the embryo-sac, while the propagating cells, egg-cells and sperm-cells have nuclei with the constant number of chromosomes characteristic of the species. We may here pass over the fact that certain writers believe to have found, in some few isolated instances, aberrant numbers of chromosomes even in propagating cells.

The importance of the number of chromosomes in species does not lose its interest in this account. On the contrary, it is a matter for surprise that plants and animals, despite the fact that deviation may take place in the "less important" tissues, are nevertheless characterized by a constant number of chromosomes in the nuclei which are exclusively of direct importance for the continued existence of the species.

Before passing on to consideration of the number of chromosomes in plants which I have myself investigated, I purpose to touch on the question of occurrence of chromosomes in the vegetable kingdom seen from a phylogenetic point of view.

As long as we are concerned with the higher plants, the nuclei and their component parts are on the whole more or less uniform in appearance. The chromatic parts are to be found, when the nucleus is in a resting position, as a rule within a nuclear wall, and on division of the nucleus, chromosomes appear, which are longitudinally divided, each of the daughter nuclei then receiving one half of each chromosome thus divided. The same thing is still found, without essential alteration, in most algæ and fungi, but in the lowest plant groups, especially schizophytes, flagellates and the simplest *Phycomycetæ*, the nucleus either differs in structure or does not exist as such.

This in itself might suggest that the nucleus with its chromosomes is an element of phylogenetically later origin than the cell, and as a matter of fact, various investigations have shown that the chromosomes in the most primitive organisms are less inseparably connected with a nucleus than in more highly developed forms.

In the *Plasmodiophoraceæ*, as also in other, more animal protozoa, the nuclear membrane often disappears for a considerable time; indeed, in *Sorosphæra Veronicæ*, Maire and Tison (1909) with Blomfield and Schwartz (1910) have found — as already observed by Nawaschin (1899) and Prowazek (1905) in *Plasmodiophora Brassicæ* — that the nuclei of multinucleate myxoplasms are entirely dissolved, new ones being then formed on the commencement of the sprogenous phase (the akaryotic stage). Albeit my own earlier investigations (Winge 1912) did not appear to me to indicate any total disappearance of the nucleus, it was nevertheless beyond doubt, that the greater part of the chromatin content in the nuclei was distributed in a most remarkable manner throughout the surrounding cytoplasm, and that the nuclear membrane was for a long time entirely dissolved. No such striking interaction between nucleus and cytoplasm is to be found in higher organisms, but it is evidently common also in holocarpous *Chytridineæ* and flagellates.

An interesting observation in this connection is, I consider, the fact that the nuclei of the *Plasmodiophoraceæ* during the anaphase of the vegetative cell division leave the old nuclear membrane and form a new one — sloughing a skin, as it were — (Nawaschin 1899; Winge 1912) the spindle in the sporogenous phase being intranuclear.

In the low form *Entorrhiza Raunkiæriana* also, which, as far as can be said at present, appears to rank among the simplest *Ustilagineae,* I have (Ferdinandsen and Winge 1914) had occasion to notice a fungus which, at any rate during spore formation, presents a remarkable cytologic picture, as will be seen from our

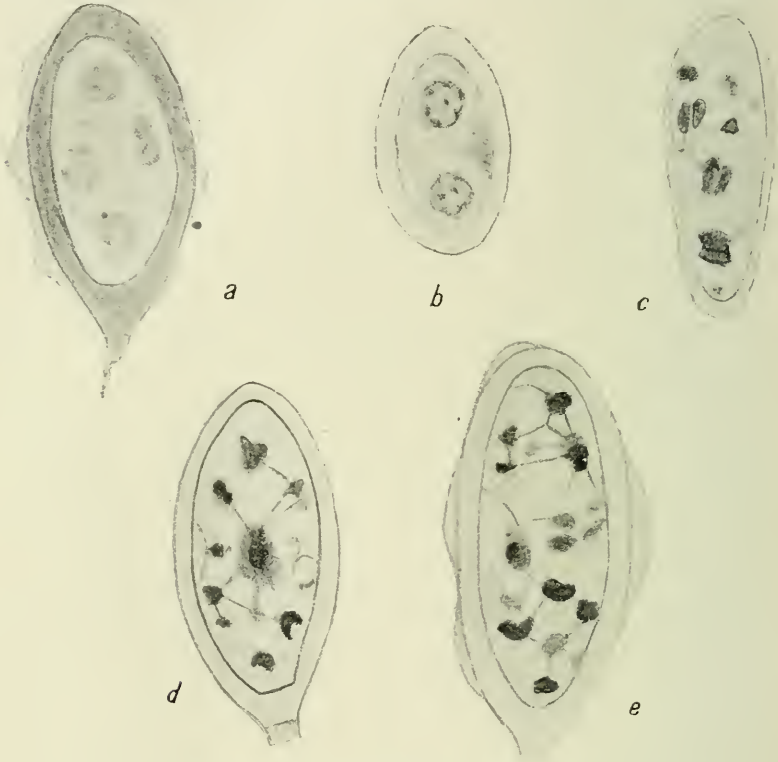


Fig. 5. Extranuclear chromosome-like bodies, partly split longitudinally, in young spores of *Entorrhiza Raunkiæriana.*

illustrations, here reproduced (Fig. 5) together with new ones (Fig. 6). On going through my preparations again, I found reason to revise my opinion in some degree as to the cytology of these spores, which unfortunately. owing to the small dimensions of the young spores, and particularly of the hyphæ, still leave much to be desired with regard to certainty as to their mode of formation. — As shown by Ferdinandsen and the present writer, the spores of *Entorrhiza* appear as a rule to arise from the union of two hyphæ, whereby in all probability a synkaryon corresponding to that of the *Ustilagineae* is formed. In any case, two fairly distinct

nuclei may often be discerned in the young spore (Fig. 5, *b*) but the presence of 3 or 4 has also been observed. At a later stage, we now encounter the peculiar feature that the cytoplasma of the spore entirely takes over the chromophile substance (Fig. 5, *c*, *d*, *e*) while a single nucleus (fusional nucleus) of amoeboid appearance, i. e. with pseudopodium-like prolongations, and entirely devoid of chromatin, remains lying in the central region of the spore (Fig. 6). In the previous investigation, I had not altogether realized that the greyish central body in Fig. 5 *d* was a kind of nucleus, and that such are always present, albeit often hardly discernible owing to its achromatic nature. I am now, however, convinced that this is the case. We here find then, in as marked a degree as in the *Plasmodiophoraceæ*, a nucleus without any content of chromatic matter, and a cytoplasm containing chromatin. Furthermore, the chromatin is here, as shown in Fig. 5 *c*-*e*, so distinctly differentiated into bodies, that one is almost tempted to call them chromosomes. The somewhat oblong chromatin bodies divide longitudinally (Fig. 5 *c*, 6). the parts remaining, at any rate for a time, connected by hyaline filaments, which may be compared with the linin threads of the karyokinetic spindle. The chromatin bodies are not present in any definite number, being fewer in the young spore, and then dividing — often several at a time — longitudinally, so that the older spore contains about 30. The spore then evidently enters upon a resting stage, as the chromatin bodies grow distinctly paler, whatever colouring matter be applied, and finally become altogether invisible. The central nucleus is then also at the best but faintly discernible.

Fig. 6. Chromatin-free nucleus in young spores of *Entorrhiza Raunkiæriana*. Some of the extranuclear chromosome-like bodies split longitudinally.

The fact that the central nucleus is altogether devoid of chromatin makes it unreasonable to identify the bodies referred to with chondriosomes or similar elements. True, we have up to the present not been able to follow the further course of the chromatin bodies, but it is nevertheless beyond doubt that in the present form, they must be homologous with chromosomes.

We find then, in lower organisms, that the nucleus is not morphologically so well bounded an organ as in higher plants. In these latter, as we know, the nuclear membrane is dissolved only for a short time during karyokinesis; the nucleus persists, and always contains chromatin. As the "cell" in several, especially primitive organisms, e. g. amoebæ, is unwalled, as wall formation must be considered to be a morphologically and physiologically higher stage, which in multicellular plants is closely connected with the division of function, so also the facts indicate that the nucleus is phylogenetically of later origin than the live cell itself, and an indication of greater physiological differentiation within the same.

It is no part of my plan to go closely into the cytological conditions in the many lower plant types with their various forms of cells, and the mentioned types, *Plasmodiophoraceæ* and *Entorrhiza*, I have selected merely as furnishing good examples of forms deviating from those of the higher plants, and lying within the range of my own experience.

A more specialized description of the state of chromosomes in the different plant groups is given in the recently published work by the eminent cytologist Tischler (1915), and I may therefore content myself with referring to this.

In conclusion, then, we can at least sum up by saying that the nucleus and chromosomes in the lowest forms are less sharply differentiated than in those more highly developed, and that the chromosomes in the latter, on the other hand, are particularly characteristic, easily recognisable and constant elements.

CHAPTER 2.

THE SYSTEM OF CHROMOSOME VALUES IN THE VEGETABLE KINGDOM.

In the course of time, as we have seen, numerous records have been published of the numbers of chromosomes in different plants, as a rule without any further speculation as to the importance of such number arising from its discovery. Only a few writers have sought to arrive at the system by which nature has accorded to the plants in question just that particular number

of chromosomes; some investigators, have, on the other hand, devoted a considerable amount of attention to this point.

As far back as 1891, Guignard observes (p. 216) that both Strasburger and himself came to the conclusion, with regard to orchids, that "il existe vraisemblablement 16 segments chromatiques dans un assez grand nombre de plantes de cette famille". And he adds: "Mais la différence qu'on observe a cet égard chez les Liliacées, entre le Lilium et le Fritillaria, par exemple, qui en possèdent 12, et l'Allium qui n'en a que 8, montre qu'il faut s'attendre à des variations dans une même famille".

It was not long before the fact was noted the pteridophytes possessed especially high chromosome values, so high that the exact figures could not be stated with certainty. The number of chromosomes in some pteridophytes, however, was so low as to approach those of the phanerogams. Strasburger discerned this, and states, in 1894 (p. 293): "As regards the Pteridophyta, although the number of chromosomes is considerable in some of them, in others it is not greater than in the Phanerogams".

The gradually increasing number of investigations gave rise to attempts at more general conclusions, and in particularly it was noticed, that the values for chromosomes[1]) were as a rule even numbers. Even after the beginning of the present century, however, we still find but few expressions of opinion on the point.

Miyake (1906) states quite briefly (p. 102): "Die Zahl der bis jetzt im Pflanzenreich bekannten Chromosomen ist fast immer eine gerade Zahl«.

Roth (1907) in his work on the propagation of the *Rumex* species, is more explicit; he states (p. 343): "Was nun die Chromosomenzahl an sich angeht, so lässt sich nicht sagen, ob ihr morphologisch ein Wert zukommt. Man vermag in der scheinbar regellosen Verschiedenheit noch keine Gesetzmässigkeit zu erblicken. Während Pflanzen, die systematisch unendlich weit voneinanderstehen, die gleiche Zahl aufweisen, finden sich in ein und derselben Familie, ja wie bei *Rumex* in einer Gattung ganze Reihen verschiedener Zahlen. Einstweilen lassen sich für diese merkwürdige Erscheinung noch keine Anknüpfungspunkte finden. Eigenartig und erwähnenswert scheint

[1]) Here, as throughout, I mean the number of chromosomes in the gametophyte, the haploid, x. Where that of the sporophyte is intended, this will be expressly stated.

es mir immerhin, dass bei *Rumex* nahe verwandte Arten, die sich äusserlich sehr gleichen, dieselbe Chromosomenzahl haben (i.e. 8), so *Rumex acetosa* L., *Hispanicus* Koch, *arifolius* All. und *R. nivalis* Hegetschw., während die verhältnismässig stark abweichenden Formen *Rumex scutatus* L. und *Rumex acetosella* andere Zahlen (i. e. 12 and 16) und andere Grössen der Chromosomen aufweisen. Endlich zeigen Vertreter der Gruppe *Lapathum* wie *Rumex cordifolius* Horn. und voraussichtlich noch manche andere aus dieser Abteilung die oben erwähnten starken Abweichungen (i. e. abt. 40)".

A special section within the study of chromosome numbers is formed by the investigations concerned with the doubling of the number of chromosomes. In several botanical and zoological forms, the existence of uni- and divalent forms has been discovered; i. e. the fact that certain races may have twice the number of chromosomes found in others belonging to the same species in the Linnæan sense. Well known in the field of zoological cytology is the *Ascaris megalocephala*, rendered famous by the classic investigations of van Beneden and Boveri; we have here two forms, univalens and bivalens, with 1 and 2 chromosomes respectively. In other organisms, however, the existence of trivalent and quadrivalent forms has been discovered. Él. and Em. Marchal (1907, 1909) were able at will to produce cultures of uni-, bi- and quadrivalent individuals in mosses, having succeeded in inducing protonema developement from normal moss sporogonia. As the diploid protonema proved to have the power of producing small moss plants, capable of further reproduction, the way was thus open for a continued omission of the reduction stage, whereby the number of chromosomes would be continually doubled. Tischler has further, in his interesting work on certain races of *Musa* (1910) given a fine example of plants which have, under culture, though by unknown means, apparently increased their original number of chromosomes by their own volition. The three races of *Musa sapientum*, "*Dole*", "*Radjah Siam*" and "*Kladi*" have, according to his investigations, 8, 16 and 24 chromosomes respectively. The higher values would be produced by somatic chromosome disvisions beyond the normal, or by monaster.

Also in different species of the same genus, moreover, it has been found that the chromosomes values may at times occur

in the proportion 1 : 2. This has often been taken in connection with the occurrence of apogamy, as it has been noted that apogamous species of a genus have as a rule twice the number of chromosomes found in the normally sexed species. It is but natural that this should have given rise to considerable speculation concerning the nature of apogamy. Numerous examples show, however, as is also pointed out by Strasburger (1910) and Tischler (1915) that angiosperm species may have an "augmented" number of chromosomes, without the slightest sexual deterioration. Nevertheless, on the whole, Strasburger (1905) is still right in stating (p. 53): "Jedenfalls vermeiden es die Pflanzen in auffälliger Weise, ihre auf eine doppelte Chromosomenzahl eingerichtete Generation mit einfacher Chromosomenzahl zu betreiben. Si ziehen es vor, den Reduktionsvorgang auszuschalten, falls eine apogamische Fortpflanzung erfolgen soll. Oder, wie vielleicht richtiger auszudrücken wäre, nur diese Art der Fortpflanzung ist bei ihnen anzutreffen, weil die andere, auf die halbe Chromosomenzahl eingeschränkte, sich nicht als existenzfähig erwies".

This supposition, that the number of chromosomes may at times be doubled, thus giving rise to the formation of "divalent" forms or species, has been thoroughly discussed by Strasburger in his work entitled "Chromozomenzahl" (1910). He considers, that either longitudinal or transverse division of the original chromosomes in an individual may lead to a doubling of the chromosome number, but that the former process results in a doubling of the volume of the nucleus, and also of the entire protoplast, whereas a transverse division of each original chromosome does not bring about any alteration in the volume.

In his summary on the doubling of chromosomes by longitudinal division, Strasburger states:

»Solche Vermehrung der Chromosomensätze muss auf mitotische Kernteilung zurückgeführt werden, die entweder nicht bis zur Trennung der Tochterkernanlagen fortschritten oder von einer Wiedervereinigung der Tochterkerne gefolgt wurden.

Die Vervielfältigung des Chromosomensatzes geht also von Längsspaltungen aus, die gleichwertige Produkte shaffen und die Zahl der homologen Chromosomen, bzw. auch Erbeinheiten im Kern entsprechend vermehren.

Die Wahrscheinlichkeit spricht dafür, dass der Ort eines solchen Vorgangs das befruchtete, noch ungeteilte Ei ist«.

Strasburger states his general impression regarding chromosome numbers (1910, p. 425) as follows: "Soweit meine Erfahrungen reichen, vollzieht sich im grossen und ganzen innerhalb der phylogenetischen Reihe, die von den Pteridophyten bis in die letzten Auszweigungen der Phanerogamen aufwärts führt, eine Verminderung der Chromosomenzahl in den Kernen. Es scheint daher, als wenn eine gegenläufige, sich innerhalb dieser Reihe wiederholt einstellende Vervielfältigung des Chromosomensatzes wie eine Art von Rückschritt aufgefasst werden müsste, die vielfach nachteilige Folgen zeitigt".

The question as to doubling of chromosome numbers has of late years occupied the attention of cytologists to a steadily increasing degree, and the discovery that related species may at times have one and the same chromosome number, and at others stand in the ratio of 1 : 2, has now and again led investigators to advance tentative explanations of this phenomenon.

Kuwada (1911) believes to have found in *Zea* (x = 12) two sets of chromosomes, and considers it thus as tetraploid. He states, in this connection (p. 177): "It is suggested that the production of innummerable races of *Zea Mays* has a certain relation with the duplication of chromosomes, resulting in the double number derived from an original form which had probably 6 chromosomes in reduced number".

Ischikawa (1911) who has in *Dahlia coronata* found the haploid chromosome value 16, but in several other *Dahlia* species 32, likewise considers with regard to these (p. 7) that "Es lässt sich annehmen, dass die Chromosomen des somatischen Kerns der letzteren tetraploid sind".

Bally again (1912) holds by the theory of a doubling of the chromosome value, especially in culture plants. His own investigations showed 16 chromosomes in *Triticum (Ægilops) ovatum*, as against 8 in other *Triticum* species.

We have yet to mention a quite recent statement of Tahara (1915) who records the finding of 9, 18, 27, 36 and 45 chromosomes in a series of *Chrysanthemum* species. The Japanese scientist does not say what conclusions he draws therefrom, but in any case, the *Chrysanthemum* must according to this afford a remarkably fine instance of chromosomatic polyploidy within the species of a single genus.

Strasburger wrote in 1910 (p. 407): "Aus einer einzigen

Ursprungszahl alle die in einer Pflanzenfamilie vorkommenden Chromosomenzahlen abzuleiten, geht aber, wie schon der Vergleich von *Rubus* (x = 6) und *Rosa* (x = 8) lehren, nicht an, daher mogen auf Vergleiche gestützte Schlussvolgerungen solcher Art nur in eng gezogenem Verwandtschaftskreis berechtigt erscheinen".

Tischler has, in his frequently cited work of last year, gone more closely into the question of the number of chromosomes and importance of the same than has hitherto been done by any other cytologist. He gives several hundred records of the numbers of chromosomes in the vegetable kingdom, and the numerical material is dealt with in various ways so as to afford the means o' drawing general conclusions

It will doubtless be best to give one or two quotations here in illustration of Tischler's views.

On p. 203 he states:

"Wenn wir jetzt die von uns zusammengestellte Liste der Chromosomenzahlen übersehen, so konstatieren wir auf den ersten Blick, dass sich allgemeine Gesetzmässigkeiten bisher nicht ableiten lassen. Freilich fällt es auf, dass gewisse Gruppen, wie die Asco- und Basidiomyceten durchweg Vertreter mit sehr geringen Chromosomenzahlen besitzen und dass auch den Moosen und den Gymnospermen im allgemeinen niedere Zahlen zukommen. Aber bei den Algen, Pteridophyten und Angiospermen haben wir neben wenigchromosomigen auch oft »unmittelbar« hochchromosomige Species, und wo, wie bei Ophioglossaceen, Equisetaceen und Lycopodiales vorläufig nur Arten mit besonders viel Chromosomen bekannt geworden sind, da werden wir durch die äusserst geringe Anzahl der cytologisch studierten Species gewarnt, bis auf weiteres unsere Erfahrungen zu sehr zu verallgemeinern".

(Page 204): "Gehen wir auf die einzelnen Familien der bisher studierten Klassen ein, so bemerken wir, dass meist einzelne Arten höhere Chromosomenzahlen, andere wieder niedere haben und wieder andere »in der Mitte« stehen. Von manchen Seiten ist darauf hingewiesen, dass Gruppen mit annähernd konstanten Zahlen, wie die Gymnospermen, die vielfach die Zahl 12 auch bei den heterogensten Gattungen haben, »stabil gewordene« Gruppen darstellen, während andere wie die Liliifloren oder die Compositen sich gegenwärtig in einer »Periode der Artbildung« befinden. Solche Abstraktionen mögen vielleicht gute Richtlinien für weitere Arbeit sein. Ihr augenblicklicher realer Wert ist aber

wohl noch sehr problematisch (vgl. jedoch die anregenden Ausführungen von Strasburger 1910).

Also mit dem Anschneiden der grossen phylogenetischen Probleme auf der Basis der Chromosomenuntersuchungen ist est wohl noch zu früh. Wohl aber meine ich, dass wir auch jetzt schon die Chromosomenzahlen verwerten können, wenn wir die Gattungen oder gar die Species mit verschiedenen Zahlen ins Auge fassen".

(Page 219): "Die Chromosomenforschung vermag uns aber auch noch von Nutzen zu sein, wo eine experimentelle Forschung bisher versagt, dann nämlich, wenn es sich um zwei oder mehrere systematisch weiter entfernte Species oder gar Gattungen handelt. Damit kommen wir zu einer Verknüpfung der Chromosomenforschung mit der »vergleichenden äusseren Morphologie«. Noch vor gar nicht langer Zeit wäre ein Inbeziehungsetzen dieser zwei Disziplinen als absurd angesehen worden. Auch jetzt liegt es noch nicht viel Positives vor".

As Tischler further observes, the number of chromosomes has of late years been employed in systematics by zoologists, who believed to have discovered that the *Cyclopidæ* were, according to their higher or lower degree of developement, characterized by lower or higher numbers of chromosomes respectively. If such a view should really prove justified, we should certainly have an extremely close connection between the chromosomes and the morphology of species!

— Passing now to consideration of the actual numbers of chromosomes in the vegetable kingdom, I will finally join with Häcker (1907) and Tischler (1915) in calling to mind that recent cytological investigations have led to the view that the numbers of chromosomes in plants and animals generally belong to the 2-figure system; 2, 4, 8, 16, etc. (i. e. values of the formula 2n) or the combined 2 and 3 figure system: 6, 12, 18, 24, etc. (i. e. terms of the formula 6n). In addition, there are a number of deviations from these, and of late years especially a considerable quantity of odd numbers have been discovered, the occurrence of which would seem necessarily to imply an occasional halving of previously existing values divisible by 2.

Statistics of hitherto known numbers of chromosomes should, as already mentioned, presumably afford a sound foundation for research directed towards the system of such numbers in the vegetable kingdom, and I have accordingly collected, from extant works, several hundred records for the values in question. Only a part of these statements were given by the respective investigators as altogether certain, and only such could here be accepted without reserve. In many cases, a first statement as to the chromosome number of a plant has been contradicted by the subsequent researches of others, or even by the original writer, after revision of his own previous work. In the case of certain plants, only the number of chromosomes in the sporophyte has been determined (to these may be reckoned the apogamous plants) — and, as noted in the foregoing, we are not justified in drawing conclusions from these as to the values for the sexual cells. Add to this the fact that some plants are stated as having varying numbers, and others are hybrids for which special conditions prevail, and we have then left but a small number of plants with chromosome values generally accepted as correct.

The advantage of most statistics is, that the few rare or abnormal cases are lost in the mass, and do not prejudice the whole. Inaccuracies tend now in the one direction, now in the other, and will on the whole cancel out. In employing statistics for such a purpose as the present, however, we have to face a different and less convenient state of things. We are here no longer concerned with the mean value of all records given, but with the distribution of the variates, and it is therefore of supreme importance that each individual figure be correct. — As everyone knows, the houses on the right side of our streets are allotted even numbers; statistical treatment of numerical material consisting of house numbers from the right side of streets would soon reveal the fact that such was the case. If, however, by mischance even a few of numbers were noted 1 too high or too low, the whole statistical computation would go to pieces, and the rule as to even numbers would not make itself apparent.

Unfortunately, the mere fact that a first statement as to the number of chromosomes for one or another plant has passed unchallenged affords no guarantee that such statement is correct. Even if we cautiously discard all records which there seems but the slightest reason to doubt, we are still not altogether on the

safe side. And here we must further reckon with a psychical element of great importance. There is a general inherent inclination in the human mind, when dealing with numerical questions, to grasp at the "nice" figures. That this is so, is well known. And undoubtedly the even numbers, and highly divisible values, have a certain attraction in themselves, which is naturally increased when other considerations speak for their selection. Every cytologist is brought up to regard it as a fact that the chromosomes are halved before a division of the cell, and that the number of chromosomes is alternately halved and doubled during the alternation of generations; there is therefore a natural temptation to proceed along the same lines; i. e. to find highly divisible multiples of 2 throughout. With the best will in the world, investigators have not been able altogether to avoid this, and a considerable number of incorrect chromosome values have indubitably resulted, as many plants, either through the presence of an especially great number of chromosomes, or by the minimal size or awkward situation of the same, have offered particular difficulties in determination of the number, and thus led the investigator to decide the point, albeit perhaps unwittingly, according to his personal estimate.

Thus then, while thoroughly aware that the records of chromosome numbers upon which I have based the graphical presentment in pages 162-163 cannot be regarded as altogether unassailable, I nevertheless consider it beyond doubt that only a very few of them are incorrect, — and also that they truly show something of that regularity which distinguishes the numbers of chromosomes in the vegetable kingdom. Many of the plants for which chromosome values are here given, have in course of time been subjected to investigation by various investigators, who have arrived at like results; in other cases, the records are based on the researches of a single cytologist, and in the latter category especially, there may be some possibility that an error here or there can have crept in.

The graphical arrangement on pages 162-163 includes, as mentioned, only a relatively small number of the plants hitherto investigated for number of chromosomes, but 200 species being here noted; the first of an alphabetical list of over 200 species which I considered, from the literature, as safe. In order to avoid a too heterogeneous mass of material, I have refrained altogether

from including other than such statements with regard to phanerogams, vascular cryptogams and mosses. The chromosome values for fungi, for instance, I have omitted altogether, as there is still some difference of opinion concerning the homology of chromosomes in fungi with those in the higher plants. Various chromosome numbers which I have myself found in the course of my own investigations have also been omitted here, as they will be specially treated later on. The material is derived exclusively from authentic statements. I have not considered it necessary to recapitulate the names of all the 200 species, as they will be found, almost without exception, in Tischler's work (1915); I will merely observe, that all families cannot, of course, be equally represented. It is hardly likely, however, that this fact will have affected the statistics to any degree worth mentioning.

A glance at the graph on pages 162-163, were the haploid chromosome numbers are noted on the left, and the corresponding plants indicated with round marks opposite the respective numbers of chromosomes, immediately reveals certain facts which, albeit hardly be surprising to the experienced chromosome investigator, who will be aware that 8, 12 and 16 are values of remarkably frequent occurrence, nevertheless exhibit far more clearly than hitherto a characteristic distribution. In the second column of the table, the chromosome numbers have been resolved into prime figures, and we here find, that there is ever a relative maximum to be found opposite the most highly divisible values, although, in an absolute sense, there is on the whole a decreasing number of species below 8 and above 12. The two first figures which can be resolved into two prime factors are 4 ($2 \cdot 2$) and 6 ($2 \cdot 3$) both of which are relative summits. The two first figures resoluble into 3 prime factors are 8 ($2 \cdot 2 \cdot 2$) and 12 ($2 \cdot 2 \cdot 3$), which are not only relative maxima, but which appear to contend for the rank of absolute summit in the entire variation curve[1]). The two first figures having 4 prime factors, 16 ($2 \cdot 2 \cdot 2 \cdot 2$) and 24 ($2 \cdot 2 \cdot 2 \cdot 3$) are conspicuous relative summits, and the same applies to the only chromosome number in the table having 5 prime factors, i. e. 32 ($2 \cdot 2 \cdot 2 \cdot 2 \cdot 2$).

[1]) Strictly speaking, of course, it must from the nature of the case be incorrect to employ terms from variational statistics in the usual manner when dealing with the present material; but we may doubtless be permitted to do so in a preliminary explanation.

Haploid Chromosome numbers	Chromosome numbers resolved into prime factors	
36	2 · 2 · 3 · 3	o o
35	5 · 7	
34	2 . 17	
33	3 · 11	
32	2 · 2 · 2 · 2 · 2	o o o o
31	31	
30	2 · 3 · 5	
29	29	
28	2 · 2 · 7	
27	3 · 3 · 3	o
26	2 · 13	
25	5 · 5	
24	2 · 2 · 2 · 3	o o o o o o
23	23	
22	2 · 11	
21	3 · 7	o o
20	2 · 2 · 5	o
19	19	
18	2 · 3 · 3	o o o
17	17	
16	2 · 2 · 2 · 2	o o o o o o o o o o o o o o o o o o o o o o o o
15	3 · 5	
14	2 · 7	o o
13	13	
12	2 · 2 · 3	o o o o o o o o o o o o o o o o o o o o o o o o
11	11	
10	2 · 5	o o o o o o o o o o
9	3 · 3	o o o o o o o o o o o o o o o o o
8	2 · 2 · 2	o o o o o o o o o o o o o o o o o o o o o o o o
7	7	o o o o o o o o
6	2 · 3	o o o o o o o o o o o o o o o o o o
5	5	o
4	2 · 2	o o o o
3	3	o
2	2	
1	1	

:EQUENCY OF CHROMOSOME NUMBERS.

0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0

0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0

Besides the figures noted, 4, 6, 8, 12, 16, 24 and 32, it will further be seen that also relatively many plants have chromosome numbers situate at 9, 18, and 36, i. e. likewise highly divisible multiples of 2 and 3.

Only 5 chromosome numbers are represented by more than 10 "variates", to wit:

Chromosome number	6	(2 · 3)	with 16	species
—	8	(2 · 2 · 2)	- 53	—
—	9	(3 · 3)	- 15	—
—	12	(2 · 2 · 3)	- 52	—
—	16	(2 · 2 · 2 · 2)	- 21	—

These five values must thus, on the basis of the present material, be regarded as the chromosome numbers most frequently occurring in the higher plants, while of these again, 8 and 12 are far more frequent than the remainder.

The five most frequent chromosome numbers, 6, 8, 9, 12, and 16, are composed entirely of the prime factors 2 and 3, and the same applies to all the chromosome values with marked relative maxima in the graphical table. This is a most interesting point, and calls for explanation.

I have already mentioned, that as long as investigations into the importance of chromosome numbers were in their very earliest stage, it would be impossible to say what determined the number of chromosomes in the organism. It would seem just as reasonable to suppose, for instance, that it should be dependent upon the size of the plant, as upon its duration of life, or other ecological conditions, or on the volume of its cells, its particular phylogeny, or anything else.

Cytologists have now learned, that the question is not an easy one to answer. It will not be necessary, however, to go through all the numerous examples which serve to prove, for instance, that the total volume of a plant is not of decisive importance for the number of chromosomes. Many small plants have a higher number of chromosomes than other growths of the size of trees, while on the other hand, larger plants frequently have a greater number chromosomes than others of smaller growth. There is thus no hard and fast rule in this respect — and the same would seem to apply, in all essentials, to the other casually selected principles for classification. It will here suffice to note the fact.

The only generally applicable rule remaining is that which has already been mentioned by several writers; to wit, that certain large systematic groups appear to be distinguished either by especially low or by especially high numbers of chromosomes, e. g. *Fungi* and *Hepaticæ* on the one hand, in contrast to *Filicales*, *Equisetales* on the other; and also, that the same number of chromosomes often occurs in several species of one and the same genus, as we have seen in the quotations from the works of Roth (1907) and Tischler (1915) (see pp. 153 and 157).

The preliminary results, although their value may appear but slight, yet prove that there is to a certain extent a connection between the number of chromosomes in plants and their systematic position. It remains for us to go further into this question.

Tischler (1915), as quoted on p. 157, expresses the opinion that it should be possible to arrive at considerable results by comparing the numbers of chromosomes within narrowly restricted systematic groups; i. e. mainly by comparison of the various species in a genus, or genera in a family. His view is, however, on the whole, that some species within a generic field have higher, others lower values for number of chromosomes, while some again are "in der Mitte"; certain genera, on the other hand exhibiting more "stability" in this respect.

He mentions 64 genera of plants, in which he has, from a study of the literature, found varying numbers of chromosomes for the different species. Tischler does not, however, mention any close inter-relation between the various numbers of chromosomes, save in the case of his own researches with *Musa sapientum*, which he has himself investigated, and *Wikstroemia Indica* and *W. canescens*, with 9 and abt. 27 chromosomes respectively. These lead him to discuss the possibility of occasional dispermatic fertilization, an interesting point to which I shall refer later on.

I am, however, of opinion, that we can, by taking due regard to the numbers of chromosomes actually found in plants, draw considerably more far-reaching conclusions than Tischler has done. Various instances will serve to illustrate this, and I may here at once intimate that I purpose to show, that related species have related numbers of chromosomes: "related" in the last case being taken to mean standing in a simple numerical relation one to another. Exceptional deviations from this rule will be dealt with in another chapter.

Plunging then at once into an apparent confusion of chromosome numbers, we may commence with the family of the *Compositæ*, which is as a rule considered as being in a marked degree unstable with regard to number of chromosomes (cf. quotations from Tischler p. 157). We would, however, reserve the right to exclude the *Cichoriæ* from present consideration, this group comprising various species where abnormal sexual conditions prevail, as for instance species of *Hieracium* and *Taraxacum*. For the same reason, the genus *Antennaria* is likewise here omitted. We have then remaining the following:

		Chromosome number		
A	Achillea millefolium	24 (?)	Lundegårdh	1909
A	Anthemis tinctoria	9	»	»
H	Calendula officinalis	14	»	»
H	» spec.	16	Rosenberg	1904
A	Chrysanthemum arcticum	45	Tahara	1915
A	» carinatum	9	»	»
A	» coronarium	9	»	»
A	» Decaisneanum	36	»	»
A	» Japonicum	9	»	»
A	» lavandulaefolium	9	»	»
A	» leucanthemum	18	»	»
A	» Marchallii	9	»	»
A	» morifolium	27	»	»
A	» Nipponicum	9	»	»
H	Dahlia coronata	16	Ishikawa	1911
H	» gracilis (?)	32	»	»
H	» Juarezii	32	»	»
H	» variabilis	32	»	»
H	Helianthus annuus	16 (?)	v. Bönicke	1911
A	Matricaria chamomilla	9	Lundegårdh	1909
H	Silphium integrifolium	8	Merrell	1900
H	» laciniatum	× 8[1])	Land	1900
H	» terebinthinaceum	× 8	»	»
A	Tanacetum vulgare	9	Rosenberg	1905

[1]) In accordance with Tischler (1915) I have here and in the following placed a cross (×) before the chromosome numbers which are derived from the diploid number; in other words where the haploid chromosome number has not been directly observed.

At a first glance, the only immediately conspicuous features will possibly be the remarkably interesting figures for the *Chrysanthemum* species, in which, as shown by Tahara, the chromosome numbers 9, 18, 27, 36, and 45, all simple multiples of 9, are the only ones hitherto found. Tahara's work is the first to give an extensive investigation of many species in the same genus, made specially with a view to the study of chromosome numbers, and will thus, always supposing the results to be correct, continue to rank as a pioneer work, despite its concise form. The investigations with *Dahlia* suggest that exactly similar conditions here prevail.

This is also, perhaps, the only instance of regularity directly noticeable; on dividing up the plants into groups according to their natural generic relations, however, we find a different state of things. The list includes only genera belonging to the *Astereæ* group, which, as we know, can again be subdivided into several groups, of which only *Anthemideæ* and *Helianthеæ* are here represented, with 5 and 4 genera respectively. Species belonging to the first sub-group are denoted by an A before the name, those of the *Helianthеæ* by an H. We see, that the figures hitherto found for *Anthemideæ* are:

No. of species	9	1	1	1	1	1
Number of chromosomes	9	18	24 (?)	27	36	45

The numbers of chromosomes found in *Helianthеæ* are as follows:

No. of species	3	1	2	1	3
Number of chromosomes	8	14	16	16 (?)	32

From this we may presumably conclude that the *Helianthеæ* are characterized by chromosome numbers which are multiples of 8, while those for the *Anthemideæ* are constantly divisible by 9. The only deviation from the rule is, in the first case, a doubtful 24, and in the second, the occurrence of 14 as the number of chromosomes. The 24, which is given by the finder with some uncertainty, we are obviously not obliged to consider, and as to the 14, though drawn from so competent an authority as Lundegårdh, I cannot but regard this as due either to an abnormity in the plant individual, or more probably to an error in counting. That such errors may occur, even in the work of

the most experienced cytologists, has been sufficiently proved in the course of cytological research, and it is far more a matter for surprise that often high chromosome numbers are given with apparently full certainty, than that the count may be incorrect also when dealing with lower figures.

We need not at present go farther into an explanation of these conditions; it will suffice to note, that even if up to now 2^3 appears to enter as a factor into all the chromosome numbers for *Helianthea*, and 3^2 on the other hand into those for *Anthemideæ*, it is yet possible that lower figures may be found in both sub-groups; say 4 in the first group and 3 or 6 in the latter. Such discovery, however, if made, would not disturb to any great degree the nice regularity now so distinctly apparent. Meanwhile, we may be justified in characterizing the respective systematic groups by the presence of 8n and 9n chromosomes respectively, 8 and 9 being designated as the cardinal numbers. On practical grounds, therefore, I propose to employ the following brief formula as meeting the case:

Heliantheæ: $x = 8n$
Anthemideæ: $x = 9n$

When the cardinal numbers for two systematic groups of which we wish to compare the chromosome numbers are so unlike as to have, as here, no prime factors in common, it will of course be easy to determine whether the regularity which I believe to be present actually is so. It is a more difficult matter when the cardinal numbers are related; i. e. have one or more prime factors in common. Had we had, in the mentioned groups, for instance 8 and 6 instead of 8 and 9, we might reasonable expect to find the chromosome numbers in series 8, 16, 24, 32, and 6, 12, 18, 24. The chromosome number 24 could occur in both groups, as being the common denominator of 6 and 8, and equally obtainable from $8 \cdot 3$ or from $6 \cdot 4$.

In accordance with the comparatively slight variation in magnitude of the number of chromosomes in plants, we find that many species having systematically nothing whatever in common, yet exhibite the same value for number of chromosomes. In investigating the regularity prevailing therefore, it will be necessary first and foremost to consider such groups as have chromosome numbers of comparatively rare occurrence — or, in making

comparison between the chromosome numbers of two groups, to select such as have rare numbers at least in the one group.

On comparing, for instance, the chromosome numbers in *Liliaceæ* with those of *Convallariaceæ* — that the latter are often included by systematists under the former is here of minor importance — we find, that the *Liliaceæ* nearly always have 8, 12 or 24 chromosomes, while the *Convallariaceæ* have 6, 12, and 18. It would therefore seem probable that the chromosome numbers for *Liliaceæ* are built up on the basis of 4, those of the *Convallariaceæ* from 6, but the occurrence of 12 in both groups detracts somewhat from the value of the example as matter of proof, and I therefore refrain altogether from going further into the chromosome values of these otherwise so extensively investigated families; the more so since one or two erroneous figures might under such circumstances easily upset the entire calculation.

Groups having rare chromosome numbers are from the nature of the case but few, but the investigations hitherto made show that where such do occur. they are generally to be found in two or more related species. Thus the figures 7 and 14 are characteristic for the hitherto investigated *Oenothera* species, and for the segregates of *Oe. Lamarckiana* (Gates 1908 a and b; Davis 1909, 1910 etc.) and a 9 system appears to prevail among the *Thymæleaceæ*. Of this last family, the following plants have been examined:

	Chromosome number		
Daphne alpina	9	Strasburger	1909
» Kiusiana	9	Osawa	1913
» mezereum	9	Strasburger	1909
» odora (highest no. found)	14	Osawa	1913
» pseudomezereum	9	»	»
Gnidia carinata	9	Strasburger	1909
Wikstroemia canescens	9	»	1910
» Indica (apogam)	26	Winkler	1906
» »	c. 26	Strasburger	1910

Six species, divided among 3 genera, have thus 9 chromosomes. The figure 14 for *Daphne odora* does not seem to be altogether certain; Osawa merely states that 14 is the highest value he has observed. Finally, we have 26 noted as being the most frequently occurring figure in the apogamous *Wikstroemia*

Indica. while on the other hand 22-29 may occurr. These figures might well seem to suggest that the original number in *W. Indica* was 3×9.

The *Solanaceæ* hitherto investigated are six in number: viz:

	Chromosome number		
Datura stramonium	12	Bönicke	1911
» » var. tatula	12	»	»
Hyoscyamus albus	$\times$ c. 18	Bonnet	1912
Nicotiana tabacum	24	White	1913
Solanum lycopersicum	12	Winkler	1910
» nigrum	36	»	»

The cardinal number here appears to be 6, with at least 2n present.

The Polygonaceæ should also be mentioned here; as already noted, Roth (1907) has investigated a number of *Rumex* species. The total chromosome numbers known are as follows:

	Chromosome number		
Fagopyrum esculentum	8	Stevens	1912
Rumex acetosa	8	Roth	1907
» acetosella	16	»	»
» arifolius	8	»	»
» cordifolius	c. 40[1])	»	»
» Hispanicus	8	»	»
» nivalis	8	»	»
» scutatus	12	»	»
» verticillatus	(probably) 24	Fink	1899

Roth's statement (see quotation p. 153) that we thus in *Rumex* find "ganze Reihen verschiedener Zahlen" may to a certain extent be admitted; on the other hand, it should not be forgotten that these figures are more or less closely related. Even including the values given by Stevens and Fink, they are still divisible by 4.

I may here also briefly refer — chiefly in order to have the opportunity of correcting one of my own earlier statements —

[1]) Tischler (1915) quotes "ca. 20", but from the statement in Roth l. c. p. 342 there can be no doubt that he means 40. Roth, however, in his illustration Pl. I Fig. 8, to which he himself refers, shows only 38!

to the little family of *Cannabaceæ*, now perhaps more generally included under *Moraceæ* or *Urticaceæ*. We are here concerned with but two genera; *Humulus* and *Cannabis*. In my work on the cytology of *Humulus* (1914) which appeared almost simultaneously with that of Tournois (1914) on the same subject, I noted the occurrence of 8 and 10 chromosomes in *Humulus Japonicus* and *H. lupulus* respectively, whereas Tournois believed to have found 10 in both. A subsequent examination of my preparations and the production of new has since convinced me that more than 8 chromosomes may be found in the Japanese Hop, and I have even been able one occasion to discern the presence of 11 chromosomes at the one pole of a heterotypical spindle. The postscript in my paper to the effect that 8 chromosomes would doubtless prove to be the correct number I must therefore retract, the more so since what I have seen in other plants has taught me that related species rarely have chromosome numbers so distantly related as 8 and 10. Tournois is undoubtedly right in giving 10 as the normal number of chromosomes for the species; on the other hand I incline to the opinion that abnormities in this respect — as also in others with regard to *Humulus* — are frequent. In *Humulus Japonicus*, the nuclear plates of the pollen mother cells are so irregular that it is difficult to count the chromosomes at all, despite their size. In *Cannabis* also, 10 chromosomes are found (Strasburger 1910 b) so that we have here again an instance of uniformity in the number of chromosomes between related forms — and in addition, a number of rare occurrence.

A recapitulation of the numbers of chromosomes in all plant families would be superfluous. Tischler's work (1915) gives an extremely comprehensive survey of the figures hitherto known. And from this, in my opinion, it will be clearly seen that most families are characterized by quite definite series of values for the number of chromosomes, such as I have quoted in the instances given above. Now and again we may encounter an apparent deviation from the rule, but the cases where such are undoubtedly known to occur are relatively very few, and most will probably be found to be due to erroneous counting, or to abnormal material. As we have just seen, in the case of *Compositæ*, two series of chromosome numbers may at times occur in a single family; in *Rosaceæ*, for instance, nearly a score of species

belonging to the genera *Alchimilla. Crataegus, Mespilus, Potentilla* and *Rosa* have chromosome numbers 8, 16 or 32, while of the genera investigated, only *Rubus* (3 species) has x = 6.

From the facts as noted, then, I must entirely agree with Tischler in his statement (quoted p. 158) that we cannot yet lay down the main phylogenetic lines on the basis of chromosome numbers. On the other hand, it will have been seen from the foregoing, that even our present knowledge will suffice to show so high a degree of regularity for the chromosome numbers within the narrower systematic groups that further study should in course of time furnish a sound foundation — even for the systematists.

CHAPTER 3.

SOME NEW CHROMOSOME NUMBERS.

As pointed out in the foregoing chapter, it would now be of interest, as a preliminary step, to ascertain the chromosome values for a considerable number of species within a generic field where more or less rarely occurring figures are found. Such an investigation should, furthermore, include several genera of the family concerned. Up to now, no such investigations comprising several genera have been made.

In seeking to find a good subject for such investigations, I collected a considerable amount of plant material and made introductory studies as to the chromosome numbers of various plants. These gave, in a comparatively short time, encouraging results, the family of *Chenopodiaceæ* proving remarkably suited to the purpose of such investigation.

In endeavouring to ascertain the number of chromosomes in a plant, it is of importance that the organs employed for the purpose be in the proper stage of developement. And as the pollen mother cells are those best adapted for counting the chromosomes, and the phase of developement in these is often somewhat difficult to determine by macroscopic observation, it was obviously an advantage to have a plant with relatively small flowers in close groups, where practically all stages of developement might be present at the same time. Another important point was that the chromosomes should be comparatively few in

number, large and preferably short in structure; also, that the separation of the chromosomes took place with great regularity. All these qualities, which would render investigation easier, and afford the investigator unlimited certainty as to the correctness of the results, I was fortunate enough to find united in the one family referred to, the *Chenopodiaceæ*, and have I consequently devoted especial attention to these.

In the case of these *Chenopodiaceæ*, no technical difficulties were encountered in the course of the work. For fixing, I have almost exclusively employed Carnoy's chloroform glacial acetic acid alcohol solution, having found that this liquid gave the most satisfactory results. A great deal of the plant material was collected from the Botanical Garden of the University at Copenhagen, some also, however, being taken in a wild state from various parts of the country. The thickness of the microtome sections was as a rule 7 μ. For colouring the preparations, I used almost exclusively Delafield's Hæmatoxylin, which gave excellent results.

The sources of error to be considered in determination of chromosome numbers are by no means few. By restricting investigations, however, to the pollen mother cells in the anaphase of the heterotypical or homoeotypical division, such sources of error may be greatly reduced. And finally, if the investigator be content to trust only to the number of chromosomes as found in nuclear plates seen in polar view, and which have not been cut into two sections by the microtome, there will then be a considerable guarantee for the correctness of the count — always presuming that the preparations used are good in themselves. Only countings made under such conditions have I admitted as sound matter of proof. Nuclear plates viewed from the side have as a rule been touched once or twice by the microtome knife, and give only approximately accurate results, the individual chromosomes often having been cut in two. Only the presence of retarded chromosomes, abnormities in the material, accidental damage to the nuclear plates on cutting, or of the rest of the preparation — and of course errors in counting on the part of the investigator himself — need then be considered. In the *Chenopodiaceæ*, as already mentioned, reduction takes place with great regularity, so that the first source of error is reduced to a minimum. By way of meeting all eventualities in the last-named

category, I have frequently requested other persons to check my results, which, by the way, in the case of this family, were extremely clear. As proof of the correctness of my countings, I had for some time contemplated taking microphotographs of the nuclear plates and publishing the photos without retouching, but as these would after all only prove that the number of chromosomes visible in the particular instance had been as stated, and not that such was the norm for the plant in question, I

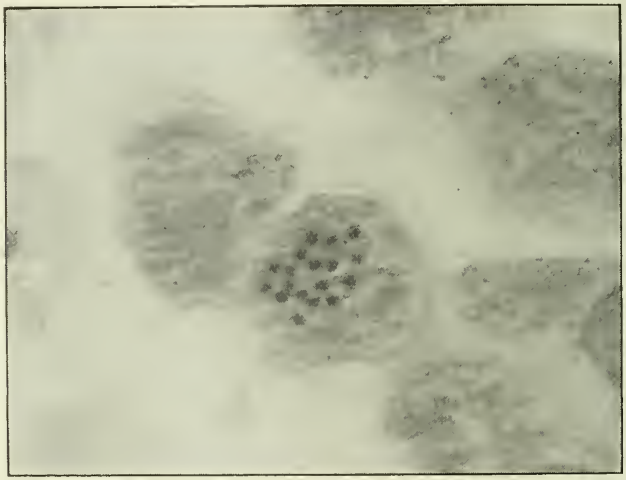


Fig. 7. *Chenopodium bonus henricus.* Microphotograph, not retouched, showing anaphase of reduction division in pollen mother cell. 18 chromosomes present.

relinquished the plan as valueless; the more so, since the technicalities of microphotography demand an aptitude in the operator which I could hardly claim to possess. I therefore give but one of the unretouched photos taken (Fig. 7). Abnormities in the material are of course difficult to guard against, but as will be seen from the results, there is no reason to believe that such should have been present in the *Chenopodiaceæ* examined.

Even where care is taken to eliminate the greatest possible number of sources of error; to use only the best optical and other aids; it will nevertheless be impossible to furnish more proof as to the correctness of the result arrived at than such as is subsequently furnished by later verification. I therefore consider it of little value to state, as has sometimes been suggested, how

many countings were made, and what number of chromosomes was found at each. Such data will never give true statistical material, as it will always rest with the investigator himself to decide which nuclear plate he will consider. In many cases a very few suitably situated will afford better proof than many less adapted to the purpose.

The numbers of chromosomes hitherto given for the family of *Chenopodiaceæ* are 3 in all, only the following species having as yet been investigated:

	Chromosome number		
Atriplex hastatum	× c. 12	Rosenberg	1909
Beta vulgaris	8	Matthijsen[1])	
Spinacia oleracea	6	Stomps	1910

These three values have little in common beyond the fact of their being all even numbers, whence the cardinal number of course, for all would be 2. Even disregarding Rosenberg's statement, which must in any case be considered as not a little doubtful, since the countings were made in the somatic tissue, there is still no greater relation between the remaining figures, 6 and 8. I shall later on have occasion to note how the cardinal number 2 in very closely related plants must at the outset suggest a doubt as to the correctness of the determinations. As, however, we are here concerned with different genera, the mentioned difference in the chromosome numbers might, a priori, well be supposed to exist, and I have as a matter of fact, here had no occasion to judge a priori, since Matthijsen's observations on the number of chromosomes in *Beta* first came to my notice quite recently through Tischler's mentioned work.

The first *Chenopodiaceæ* which I myself examined was **Chenopodium album**, of which I collected some specimens growing wild from several localities in the vicinity of Copenhagen, others from the cultivated plants in the Botanical Garden. It showed very distinctly a number of 9 chromosomes in the anaphase of the heterotypical division (Fig. 8). The chromosomes are at this stage extremely short in structure, almost spherical, and lie as a rule well apart, and in one plane, so that the counting may be made with great accuracy. The number of chromosomes was

[1]) Matthijsen's statement is published by Franck (1911) without comment.

exactly suited to my purpose, as the figure 9 contained the prime factor 3, instead of the so frequently occurring powers of 2. The figure 9 in this species led me, as indicated, to investigate several other related species and genera.

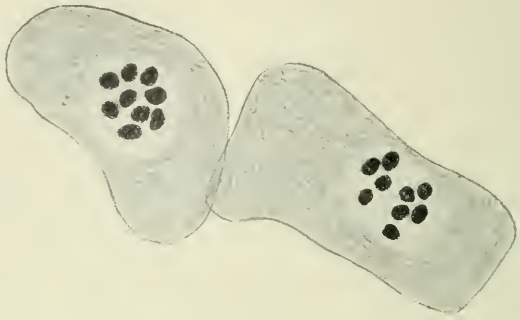


Fig. 8. *Chenopodium album.* Pollen mother cells. Anaphase of reduction division. 9 chromosomes.

Chenopodium hybridum, of which material was fixed in the Botanical Garden, likewise showed 9 chromosomes (Fig. 9). These were of the same shape as those in *C. album*, but slightly smaller in size.

Fig. 9. *Chenopodium hybridum.* Pollen mother cell. Anaphase of reduction division. 9 chromosomes.

Fig. 10. *Chenopodium murale.* Pollen mother cells. Anaphase of reduction division. 9 chromosomes.

Chenopodium murale, likewise from the Botanical Garden, had, like the two foregoing species, x = 9. The chromosomes were here distinctly smaller than those of *C. album* (Fig. 10).

That the haploid chromosome number nevertheless is not uniformly 9 throughout all species of this genus was proved by a study of the conditions in **Chenopodium bonus henricus.** Material of this species was collected partly from wild plants in North Sealand, partly from the Botanical Garden. A first glance at the nuclear divisions of the pollen mother cells at once

revealed the presence of a number of chromosomes far exceeding 9. In the diakinesis also, considerably more than 9 pairs of chromosomes were observed. Fig. 11 *a* for instance rather gives the impression that twice the number are present, since 9 chromosomes are visible in the half of the nucleus shown. As a

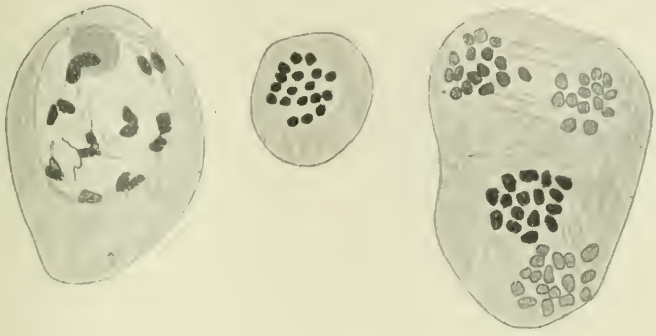


Fig. 11. *Chenopodium bonus henricus.* Pollen mother cells. *a*, Diakinesis; only one half of the nucleus visible; *b*, anaphase of reduction division; 18 chromosomes; *c*, anaphase of the homoeotypical division; several chromosomes lacking.

matter of fact, 18 is the characteristic number of chromosomes for this species, as I have definitely ascertain by repeated countings. Fig. 11 *b* shows a nuclear plate with the full complement, and Fig. 11 *c* a pollen mother cell in the anaphase of the homoeotypical division, though without its full supply of chromosomes. The chromosomes in *C. bonus henricus* are apparently somewhat more angular in shape than in the two foregoing species, but this difference is perhaps merely accidental. The microphotograph shown on p. 174 (Fig. 7) in which 18 chromosomes can be counted, is of *C. bonus henricus*.

The fifth and last species of this genus which I have had an opportunity of examining is **Chenopodium vulvaria,** which, like the first-mentioned, has 9 chromosomes of the usual

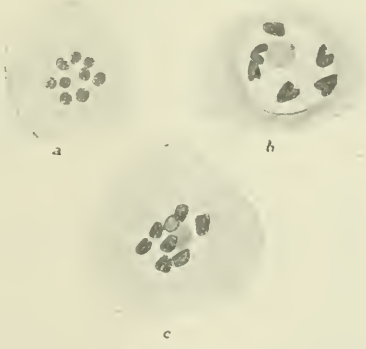


Fig. 12. *Chenopodium vulvaria.* Pollen mother cells. *a*, anaphase of reduction division; 9 chromosomes; *b*, diakinesis; *c*, stage between diakinesis and heterotypical metaphase.

appearance (Fig. 12 *a*). In the diakinesis, also we find, correspondingly, 4 to 5 pairs of chromosomes in each half nucleus (Fig. 12 *b*). An intermediate stage between the diakinesis and the anaphase of the heterotypical division is shown in Fig. 12 *c*, and the arrangement of chromosomes is here highly remarkable; at a superficial glance, only 9 are visible. On closer observation, however, it will be noticed that there is a fairly distinct split in each of the bodies, giving 9 pairs of chromosomes, but with unusually intimate pairing.

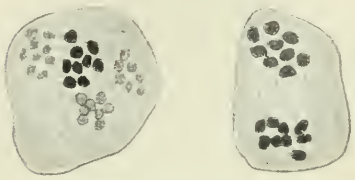


Fig. 13. *Beta vulgaris* var. *perennis*. Pollen mother cells. Anaphase of reduction division and of the homoeotypical division; 9 chromosomes.

I have thus, then, in the genus *Chenopodium*, out of 5 species examined, found 4 with 9 chromosomes and one with 18 in the haploid phase.

Beta vulgaris var. **perennis** (= *B. maritima*) from the Botanical Garden was the next plant in which I determined the number of chromosomes. Numerous sure countings proved, that 9 was again the haploid chromosome number. The chromosomes themselves were not noticeably different from those of *Chenopodium*, being, like these, somewhat rounded, and very similar in point of size. (Fig. 13).

Spinacia oleracea was collected from a cultivated bed in a garden near Aarhus. The slight number of chromosomes present leaves no doubt that Stomps' earlier statement (1910) of 6 is correct (Fig. 14). As also noticed by Stomps, there is in this plant a quite considerable difference in the size of the chromosomes. The Belgian writer informs us that 3 are larger, 3 smaller. Numerous pictures have given me the same impression. As the shape of the chromosomes here is more irregular and oblong than in the previously mentioned species of the family, it is often difficult to determine whether any constancy in such respect exists.

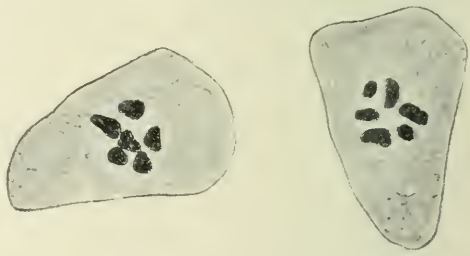


Fig. 14. *Spinacia oleracea*. Pollen mother cells. Anaphase of reduction division; 6 chromosomes of unequal size.

I have further counted the chromosomes in 3 species of the genus *Atriplex*.

Atriplex hastatum, collected from the Botanical Garden, and also from North Sealand and Jutland, had 9 chromosomes, which does not appear to agree with Rosenberg's statement (1909) of abt. 24 in the sporophytic tissue. As, however, discrepancies between the haploid number of chromosomes and that of the somatic cells is a well known phenomenon also in other plants, I need not dwell on this point. Fig. 15 *a* shows a newly formed

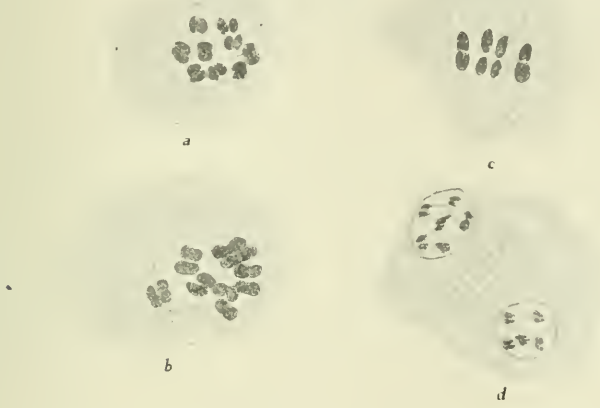


Fig. 15. *Atriplex hastatum*. Pollen mother cells. *a*, early metaphase of reduction division; 9 chromosomes. *b*, somewhat later stage. *c*, early anaphase. *d*, interkinesis.

heterotypical nuclear plate (metaphase) where the linin threads are distinctly visible (Zugfaser) proceeding from either end of the paired chromosomes. Fig. 15 *b* shows about the same stage, but this plate is viewed obliquely from above; here also the linin threads are remarkably distinct. In Fig. 15 *c*, the separation has commenced, and in Fig. 15 *d* we have an interkinesis, where the splitting for the subsequent homoeotypical division is visible.

Atriplex littorale. Specimens were examined both from Aarhus and from North Sealand, and found to contain likewise 9 chromosomes (Fig. 16 *a*). In the young pollen grains, a number of chromatin grains greater than 9 was observed; they lay for the most part arranged in pairs (Fig 16 *b*). I am unable to

explain this otherwise than as indicating the preparatory stage for subsequent nuclear division in the pollen grains.

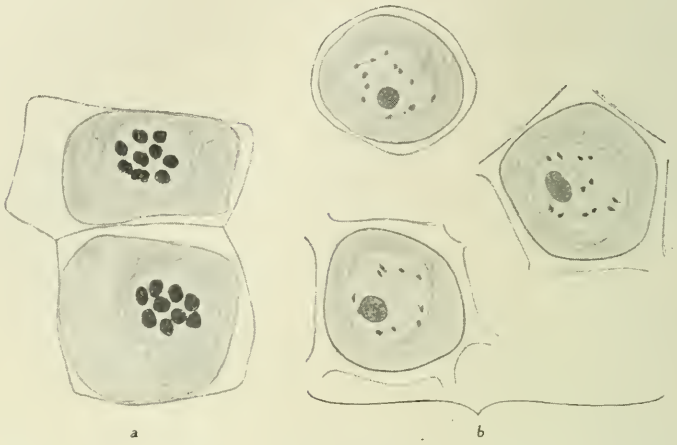


Fig. 16. *Atriplex littorale*. *a*, pollen mother cell. Anaphase of reduction division; 9 chromosomes. *b*, young pollen grains with chromatin grains partly arranged in pairs.

Atriplex patulum from the Botanical Garden finally proved that 18 chromosomes also occur in this genus. The chromosomes were particularly large, and rounded in shape, as shown in Fig. 17. In this plant also, there was not the slightest doubt that 18, neither more nor fewer, were normally present.

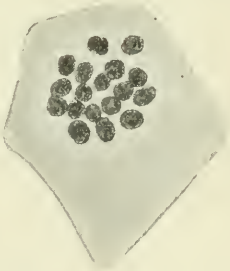


Fig. 17. *Atriplex patulum*. Pollen mother cell. Anaphase of reduction division; 18 chromosomes.

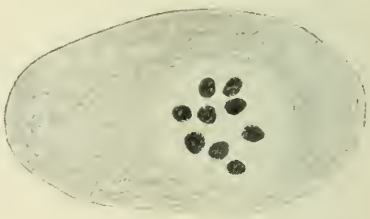


Fig. 18. *Bassia hirsuta*. Pollen mother cell. Anaphase of reduction division; 9 chromosomes.

In conclusion, I have examined **Bassia hirsuta,** the material of which I collected in the growth house of Carlsberg Laboratory. The chromosomes resemble in shape very much those of the previous genera — apart from *Spinacia* — and the haploid number was found to be 9 (Fig. 18).

I will now briefly recapitulate the chromosome numbers in the 11 species of the family *Chenopodiaceæ* which I have examined, and append therefore the following tabular survey:

	Chromosome numbers
Atriplex hastatum	9
» littorale	9
» patulum	18
Beta vulgaris var. perennis	9
Chenopodium album	9
» hybridum	9
» murale	9
» vulvaria	9
» bonus henricus	18
Bassia hirsuta	9
Spinacia oleracea	6

Albeit only 5 genera are represented, and it might be desirable to include still further types of the family, such as more particularly the genera *Kochia, Salsola, Salicornia* and *Chenopodina*, — which I hope on a subsequent occasion to be able to do — it is nevertheless evident that there is an altogether incontestable relationship between the chromosome numbers of the various species and genera. The figures 6, 9 and 18 all contain the prime factor 3, being composed of $3 \cdot 2$, $3 \cdot 3$, and $3 \cdot 3 \cdot 2$ respectively. The cardinal number for the entire family then, as far as my investigations go, is 3.

Matthijsen's statement as to the occurrence of 8 chromosomes in *Beta vulgaris* altogether disagrees with the figures which I have found to be characteristic for the *Chenopodiaceæ*. and I cannot hesitate to express my doubts as to the correctness of his results in this instance. As Matthijsen's find is not published by himself, we are unable to form an opinion as to the degree of certainty with which he himself regards it. I hope soon by further studies on my own part to arrive at a conviction regarding the chromosome number of *Beta vulgaris*. which I must a priori expect to find in agreement with *B. v.* var. *perennis*. i. e. 9, the more so since the two varieties form fertile hybrids, as I am kindly informed by Mr. E. Lindhard, leader of the experimental station at Tystofte, Sealand.

In addition to the *Chenopodiaceæ* mentioned, I have also investigated, with more or less success, the chromosome numbers of several other plants. Disregarding, for the present, all other cytological features in these, and noting merely the number and appearance of the chromosomes, I now briefly give my results.

Polystichum falcatum. Young sporangia, isolated from an indoor plant and fixed, showed a very large number of chromosomes were present, but I was unable to determine the exact number. The size of the spore mother cells involved division by the microtome into about 5 sections. As far as I could see, there

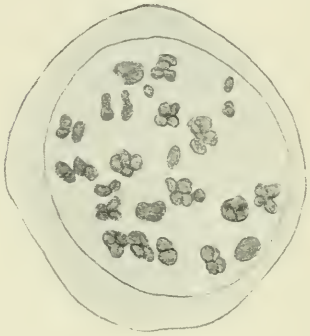


Fig. 19. *Polystichum falcatum.* Single section through pollen mother cell. Anaphase of reduction division. Chromosomes of peculiar vacuolary structure, apparantly compound.

Fig. 20. *Anemone nemorosa.* First generative division in pollen grains; 12 chromosomes.

were about 80 chromosomes, and these showed a remarkable vacuolary structure, presenting the appearance of being composite (Fig. 19). This high figure agrees very well with the chromosome numbers for hitherto investigated *Polypodiaceæ*, which vary from abt. 30-100.

Anemone nemorosa. Material was collected in North Sealand, but was at a so advanced stage of developement that only divisions of the pollen grains could be observed. Here I was able once or twice to discern the presence of 12 ribbon-shaped chromosomes of different lengths, lying extremely scattered throughout the pollen grain, with no visible nuclear membrane (Fig. 20). This number agrees well with those for the remaining *Ranunculaceæ*, among which 12 is the figure most frequently observed. The figures hitherto known for these are as follows:

	Chromosome number		
Aconitum napellus	12	Overton	1893
Delphinium Ajacis	12	Bönicke	1911
Ficaria ranunculoides	c. 6	Souèges	1913
Helleborus foetidus	12	Strasburger	1888
» viridis	12	Franck	1911
Paeonia officinalis	8	Wefelscheid	1911 [1])
» peregrina	8	»	»
» spectabilis a. o.	12	Overton	1893
Thalictrum minus	12	»	1909
» purpurascens	24	»	»
Trollius Europaeus	11—12	Lundegårdh	1914

Unfortunately, the chromosome number for *Ficaria* has not yet been determined with certainty, but as the statement of abt. 6 agrees well with the occurrence of 12 and 24, we may perhaps expect that the cardinal number in the *Ranunculaceæ* will prove to be 6, and that Wefelscheid's 8 for *Pæonia* is incorrect — the more so, since Overton has found 12 in several species examined. On the other hand, there is nothing in the values hitherto found to prevent the possibility of 4 as the cardinal number, if the 6 for *Ficaria* prove incorrect.

Chelidonium majus and **C. majus** var. **laciniatum** were collected north of Copenhagen, each in the immediate neighbourhood of the other, and showed on investigation the number of chromosomes as 6. I may here at once note, that Bönicke (1911) after examination both of the main species and the mentioned variety gives x = 8. After a thorough study of my own material, I believe I can explain how Bönicke arrived at his higher figure. The chromosomes in the pollen mother cells are of medium size, rather short, and with a marked power of dividing at a stage when such division has in other plants not yet set in. The single chromosomes are of somewhat varying size, and one chromosome in particular seems to have a tendency to split up into two. Figs. 21 *a* and *b* show heterotypic metaphases, viewed from one pole, where the division may be distinctly seen in *b*. A slightly more advanced stage is shown in *c*, where the spindle is seen from the side. Already in the early anaphase, the six chromosomes moving towards the pole are not only

[1]) If. Tischler (1915).

divided into two, but into four, thus resembling a *Sarcina* (Fig. 21, *d*, *e*, *f*), and indeed, it would even seem as if still finer subdivisions were present, which renders it difficult to ascertain the true number of chromosomes (see Fig. 21*f*). In the late telophase,

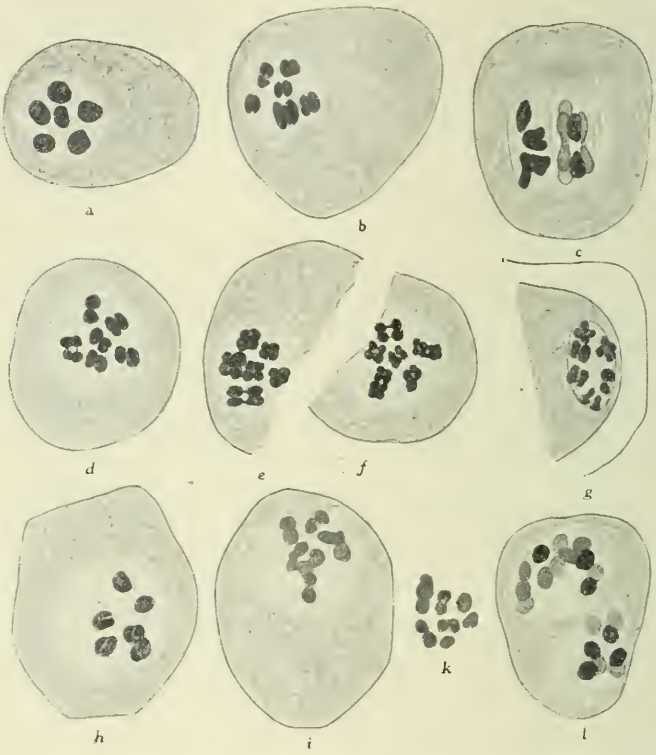


Fig. 21. *Chelidonium majus (a, b, d, e, f)* and var. *laciniata (c, g, h, i, k, l)*. Pollen mother cells. *a*, *b*, metaphase of reduction division; 6 chromosomes. *c*, transition to anaphase; *d*, *e*, *f*, anaphase; *Sarcina*-like chromosomes. *g*, *h*, telophase with split chromosomes. *i*, *k*, telophase with irregularly joined chromosome flakes. *l*, homoeotypic prophase with chromosomes already split.

the chromosomes are divided to a greater or lesser degree, the fourfold division appearing even at this stage (Fig. 21, *g*, *h*) while in other cases, the pairs of chromosomes are already separated, or somewhat irregularly paired (Fig. 21, *i*, *k*). — In the prophase of the homeotypic division, before the nuclear plates have formed, 12 chromosomes can be counted on each spindle (Fig. 21 *l*), of which 6 are attracted to either side.

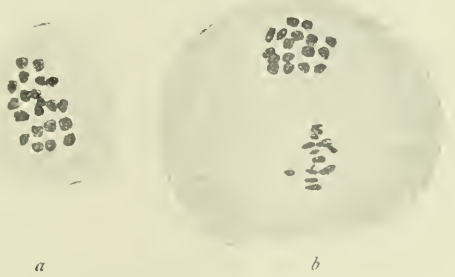
The splitting phenomenon is characteristic both for the main species of *Chelidonium majus* and for the var. *laciniatum*, but in the material which I examined, there appeared to be a greater tendency to form *Sarcina*-like chromosomes in the latter than in the main form. — The fact is of considerable interest from a theoretical point of view, as the distinct division in the heterotypic anaphase showes that the chromosome must not be regarded as an indivisible unit, but as a complexity, in which an inner differentiation or duality is already present. And in all probability, it is also actually existent even when no outward and visible sign of division is discernible

I find then, from my own investigations, that the haploid chromosome number is 6, and would at the same time explain the discrepancy between this and Bönicke's statement as due to the fact that similar divisions must have been present in his material, giving rise to the presumption that the number of chromosomes was greater.

There being, as far as I am aware, no certainly determined chromosome numbers for other *Papaveraceæ*, no comparative survey can here be given.

— I cannot pass over these phenomena of division in the chromosomes without calling to mind the recent observations of Tschernoyarew (1914) with regard to *Najas major*. This writer finds, both in the male and female plant, $2x = 14$ — one pair, however (in the soma two pairs) of chromosomes, differing greatly in size, being connected by fine threads, and fusing together during reduction, so that only 6 chromosomes appear. The occurrence of such "satellite chromosomes" will, he considers, account for the discrepancy between Guignard's and C. Müller's earlier figures, the former giving the somatic number 14, the latter the haploid, 6.

Alliaria officinalis, from North Sealand, showed a fairly high number of chromosomes (Fig. 22, *a*, *b*) but I was not able to determine the exact figure. There appeared to be about 18-20.



a *b*

Fig. 22. *Alliaria officinalis*. Pollen mother cells. *a*, anaphase of reduction division. *b*, homoeotypic metaphase.

Platanus acerifolia[1]) was collected in the Botanical Garden, and experience showed, that inflorescenses of 4-5 mm. diameter were at a suitable stage of developement. The number of chromosomes was without difficulty determinable as 21, an extremely rare figure, which invites further investigation of this plant family. The heterotypic nuclear plates were extremely fine and regular. Chromosomes isodiametric, and fairly uniform in size (Fig. 23).

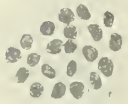


Fig. 23. *Platanus acerifolia*. Pollen mother cell. Anaphase of reduction division; 21 chromosomes.

Callitriche verna was fixed in the Botanical Garden, and investigation showed the number of chromosomes as 8. The cytological conditions accompanying pollen formation were here of so peculiar a character that I must deal with this point specially in a subsequent chapter.

Staphylea pinnata. Material was collected from a cultivated specimen in the garden of Carlsberg Laboratory. Both in the

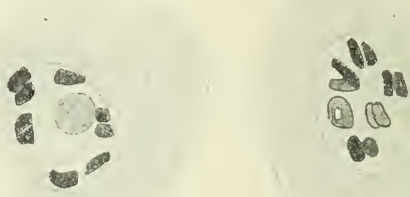


Fig. 24. *Staphylea pinnata*. Pollen mother cell in two sections. Diakinesis.

diakinesis (Fig. 24) and in the following anaphases pictures were observed which leave no doubt that there are normally 12 pairs of chromosomes present, and mitoses in the basis of the ovary revealed fairly certainly the existence of 24 in these somatic cells. Once, however, a certain x = 13 was found.

Mottier (1914) had previously given x = 36 for *Staphylea trifolia*, adding, however, (p. 126) that there will doubtless in any case not be over 40 or 42. There would thus appear to be three times as many chromosomes in *S. trifolia* as in *S. pinnata*.

Interesting in this connection are the chromosome numbers in the related family of *Aceraceæ*, of which 3 species belonging

[1]) According to the Index Kewensis = *P. orientalis*.

to the genus *Acer* have been investigated, revealing conditions apparently quite analogous with those of the *Staphylea* species; the following statements are given:

		Chromosome number		
Acer	negundo	13	Darling	1909
»	»	12 or 14	Mottier	1914
»	platanoides	c. 11	Cardiff	1906
»	rubrum	c. 36	Mottier	1914

As will be seen, the certainty of the figures is not altogether without reserve. Mottier's statement as to *Acer negundo* "12 or 14, hardly 13" and for both *Acer rubrum* and *Staphylea trifolia* "36, doubtless in any case not over 40 or 42" I can only explain as due to this writer's having allowed his evident supposition that chromosome values must be even numbers to affect the results of his actual counting, and thus coming to a sort of compromise between prejudice and experience. This is, as a matter of fact, after all, not an unpardonable fault, for what we thus may term "prejudice" is as a matter of fact frequently itself based on experience, or "supposed experience" and may be to a certain extent justifiable. It would of course be an ideal state of things if in such cases especially the subjective tendency were more strictly subordinated to the objective observation, albeit this may seem difficult, or almost impossible to attain.

Gunnera chilensis from the Botanical Garden I did not succeed in fixing at the right time, the pollen grains being already formed in my material. In these, however, a quantity of mitoses were observed, where the spireme thread was divided into abt. 12 divisions, apparently situated serially one after another, forming an endless chain (Fig. 25).

Fig. 25. *Gunnera chilensis*. First generative division in pollen grains.

Samuels (1912) has also estimated the number of chromosomes in *Gunnera macrophylla* at abt. 12, which fact I did not learn until later, from a quotation in Tischler (1915).

Hippurus vulgaris. Material from the Botanical Garden showed the number of chromosomes as 16 (Fig. 26). Juel (1911) mentions having found about the same number.

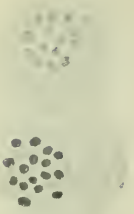


Fig. 26. *Hippurus vulgaris*. Pollen mother cell. Homoeotypic metaphase; 16 chromosomes.

Cornus glabrata. This plant gave me some difficulty in counting the chromosomes, now 11, now 12 being discernible in the anaphases of the pollen mother cells. Most frequently I found 11, but in some cases 12 were certainly visible (Fig. 27). In the metaphase of the homoeotypic division, approximately as in *Chelidonium*, a greater number may be seen, this being due to the fact that the chromosome halves are then already divided. As the presence of 12 chromosomes would a priori

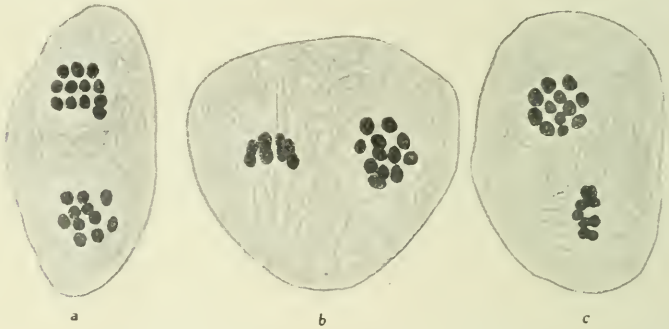


Fig. 27. *Cornus glabrata*. Pollen mother cell. Metaphase of homoeotypic division.

seem more likely than that of 11, and as the highest figure found should generally be regarded as the most correct, I must incline to the view that x = 12; the final decision, however, must be left to later investigations.

Fig. 28. *Cornus candidissima*. Pollen mother cell. Metaphase of reduction division.

Cornus candidissima, likewise investigated, showed a smaller number; here also, however, I was unable to determine with certainty from my material whether x should be = 8 or = 9 (Fig. 28, *a*, *b*).

Anthriscus silvester. Strange to say, no chromosome number has up to the present been given with certainty for the great family of the *Umbelliferæ*. And the only statement I know of at all is for this very species, *Anthriscus silvester*, by Petersen (1915). This writer mentions having found 7 and 8 chromosomes in the diakinesis of the pollen mother cells and later stages, and has also once observed 8 on formation of the embryo sac. Petersen opines that 8 should probably be taken as the correct figure, and I cannot but agree with him on this point. The chromosomes in this species are unusually large (Fig. 29 *a*) and have, as noted for several of the plants previously mentioned, the power of dividing at so early a stage after the heterotypic division that we can, in the evidently brief and not very marked interkinesis, which is roughly identical with the telophase, often count a far larger number (Fig. 29 *b*). The cytological material was taken from wild plants found at various localities in North Sealand.

a *b*

Fig. 29. *Anthriscus silvester*. Pollen mother cell. *a*, anaphase of reduction division; 8 chromosomes. *b*, telophase-interkinesis with chromosomes already split.

Fig. 30. *Ægopodium podagraria*. Pollen mother cell. Anaphase of reduction division.

Fig. 31. *Myosotis micrantha*. Pollen mother cell. Anaphase of reduction division. Extremely small chromosomes.

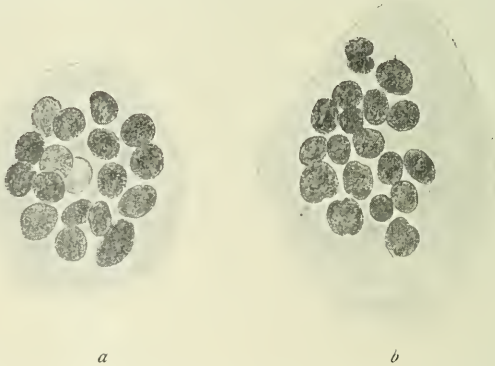
Ægopodium podagraria, likewise from North Sealand, has a considerably larger number of chromosomes, viz. abt. 20 (Fig. 30). My investigations of the species did not give any absolutely certain result.

Myosotis micrantha. No representative of the *Borraginaceae* has hitherto been investigated, and my attempt as determining the chromosome number here gave but an approximately correct result. The chromosomes themselves, however, were the more interesting, being distinguished by their extraordinarily small size; the smallest I have ever found in any of the higher plants (Fig. 31). In the heterotypic anaphase they were found to the number of abt 18-20.

Myosotis versicolor, material, like that of the foregoing, from a field in North Sealand, had more numerous and slightly larger chromosomes. The number I estimated at abt. 30 (Fig. 32). In both the *Myosotis* species investigated the picture presented by the nuclear division in the pollen mother cells gave an extremely regular and well-fixed impression, so that the difficulty in determining the number of chromosomes really must have been due to their extreme smallness of the bodies themselves. I should imagine that the uncertainty in this respect might be obviated by the use of an objective with 1.5 mm. focal width, which gives a magnifying power 33 % above the Zeiss' homog. Im 2 mm. which I employed.

Fig. 32. *Myosotis versicolor*. Pollen mother cell. Anaphase of reduction division.

Sambucus nigra, with the var. **aurea** and var. **linearis** of the



a *b*

Fig. 33. *Sambucus nigra* var. *aurea (a)* and var. *linearis (b)*. Pollen mother cells. 18 chromosomes, extremely voluminous.

same species, I have also investigated, with material collected partly from the Botanical Garden, partly from private gardens in Copenhagen. The number of chromosomes in *Sambucus nigra* is already known from the researches of Lagerberg (1909) and Bönicke (1911). Both these writers found 18, and I myself arrived at the same result, — also in the case of the two mentioned cultivated varieties. The chromosomes are, in contrast to those of *Myosotis*, extremely voluminous (Fig. 33, *a, b*). In the anaphase of the heterotypic division, a cleavage line is often observable, indicating the plane of separation for the subsequent homoeotypic division.

It is interesting to note, in this connection, that Lagerberg (1909) found the chromosome number 18 in *Adoxa moschatellina*, the relationship of which to the *Caprifoliaceæ* has been doubted. Lagerberg, who discusses in his paper the systematic position of *Adoxa*, demonstrating especially on cytological grounds its close relationship with *Sambucus*, notes among many other qualities common to the two plants the fact that both have 18 chromosomes.

Bellis perennis. In investigation of this species, the material of which was obtained from a lawn in Copenhagen, it was found that the nuclear plate in the heterotypic anaphase contained 9 chromosomes (Fig. 34). — I have already gone through the chromosome numbers in various *Compositæ*. and have shown, that the cardinal number differed in the two subgroups under *Astereæ*: *Anthemideæ* and *Helianthei*. the formula for these, according to investigations up to date, being $x = 9n$ and $x = 8n$ respectively. The finding of 9 chromosomes in *Bellis* thus affords a further instance of plants in the sub-group *Anthemideæ* with a number of chromosomes in agreement with the formula found.

Fig. 34. *Bellis perennis*. Pollen mother cell. Anaphase of reduction division. 9 chromosomes.

— On summing up the results of my own chromosome investigations, together with those already known, we find then, that Nature here proceeds according to rules which do not appear hopelessly inscrutable, and which may be put to good use in the study of plant-systematics. True, we find the same chromosome numbers — and particularly the same cardinal numbers — in widely different plant groups, and it is thus by no means possible to construct the natural plant system from a knowledge of the chromosome numbers alone. But in critical cases, when seeking to decide the question of possible relationship between minor systematic units, the chromosome number will at times afford the final weighty argument pro or contra.

CHAPTER 4.

THEORETICAL STUDIES ON THE ORIGIN OF THE SYSTEM OF CHROMOSOME NUMBERS.

With the empirical basis here given, it will now be natural to consider the chromosome numbers of plants from a theoretical point of view. Several investigators have devoted their attention to this question, and most of the theories previously advanced have been more or less intimately connected with the idea of "reduplication of chromosomes" — which as a matter of fact is hardly surprising, since in all the cells we find the chromosomes splitting up, and doubling their number. It has, moreover, long since been demonstrated that apogamous species have as a rule a chromosome number twice that of the normally sexed in the same genus, and in consequence, we find it frequently asserted that reduplication of the chromosomes often involves loss of the power of sexual propagation.

The considerations advanced in connection with these points are, as it seems to me, not altogether satisfactory, and I therefore propose in the following to go further into the question, the correct solution of which is of great importance for the whole study of chromosomes generally.

Strasburger's contributions in particular have served to popularize the theory that the higher chromosome numbers in certain "tetraploid" species should be due to reduplication of previously existing lower values. Other writers have, however, also supported this view. There is a considerable mass of literature dealing with the subject of chromosome reduplication, either in the form of works specially devoted to this particular point, or more incidentally touching upon the problem (Strasburger 1907, 1910, Rosenberg 1909, Tischler 1910, Němec 1910, Kuwada 1911, Ishikawa 1911, Bally 1912, Pace 1913, Gates 1914, a, b, and others).

In many genera such tetraploid species are known; in some few again, we find a longer series, i. e. species with 2x, 4x, 6x, 8x, etc. The investigations of Tischler (1910) with various races of *Musa sapientum* gave us the first known examples of the fact that races of a plant species may exist with three different chromosome numbers, the "*Dole*" race having 8, whereas "*Radjah*

Siam" has 16 and "*Kladi*" 24. As mentioned, Tischler considers it likely that one or two extra chromosome divisions or monaster respectively may have given rise to the increased number of chromosomes.

Here, I must confess, I am unable altogether to agree with Tischler. A doubling of the chromosome number 8 certainly gives $x = 16$, a new extra division, however, must necessarily give $x = 32$. As var. *Kladi* has but 24 chromosomes, it would then be necessary to assume that the chromosomes had redivided only in one of the daughter nuclear plates, an explanation which seems to me highly improbable.

An extra tripartition of the chromosomes in the original form with 8 would give $x = 24$; such a theory, however, is likewise altogether inacceptable, and in fact does not seem to have any supporters. No one has ever observed a threefold division of chromosomes, and the power of repeated bipartition is so characteristic for these bodies that it must be regarded as a fundamental and unalterable feature most intimately connected with the entire nature of the chromosomes themselves.

In his latest work (1915) Tischler seems more inclined to explain the origin of the mentioned *Musa* races by the theory that dispermy may have taken place, — as observed by Němec (1912) in *Gagea lutea*. The penetration of two male nuclei into the egg cell, and fertilization of the same would necessarily, provided the germ were capable of developement, give rise to a plant with $^3/_2$ the original number of chromosomes. Tischler thus considers it possible to explain the origin of a race with $x = 24$ by taking $x = 16$ as starting point. The chromosome number 8, however, cannot be brought into line with the two other figures by this method. In other words, it would be necessary to regard the higher chromosome numbers in *Musa* as arrived at by different means.

As regards the possibility of normal developement in polyspermatically fertilized organisms, it must undoubtedly be admitted that there is nothing, theoretically speaking, to prevent this from taking place under certain circumstances, though most of the experiments made in this direction, including those with animal material, do not exactly support the theory. That plants may exist with doubled number of chromosomes has been fully demonstrated by the experimental researches of Marchal and

Marchal (1907, 1909), by which, moreover, it has been further shown that the power of sexual propagation is not lost, even though the number of chromosomes be doubled. — In numerous other plant genera we may, as already mentioned, also find normally sexed species with "double" chromosome number, e. g. among the species of *Chenopodium* and *Atriplex* investigated by the present writer, with 9 and 18 chromosomes respectively. Since therefore plants with "augmented" number of chromosomes may very well exist, and even be normally sexed. there is, as mentioned, nothing theoretically to prevent the same applying to individuals produced by polyspermatic fertilization. Dispermatic fertilization must, however, often prevent subsequent normal reduction division.

For all this, however, it must be said, that the idea of the chromosome number of a species arising from doubling or trebling that of another species is in itself unreasonable. How could it be possible for forms essentially new to be produced by the mere occurrence of chromosomes — and possibly therewith genes — in twice the normal number? It might no doubt be imagined, that a reduplication of chromosomes could take place in some plant, and that this would result in the production of two races differing particularly in regard to size, and possibly incapable of interfertilization[1]). Such races might then be supposed to develope in slightly different directions, but it would seem more than doubtful whether this could be of any considerable phylogenetic importance. From the numerous investigations with *Oenothera*, and those of Marchal with *Amblystegium*, also on purely theoretical grounds, there seems no reason to suppose that the reduplication of chromosomes should in itself be of supreme importance.

How then, can these chromosome numbers have arisen? And how can it be, that a double chromosome number at times involves apogamy, at others not?

[1]) Since the completion of this work, I have received Winkler's highly interesting paper "Ueber die experimentelle Erzeugung von Pflanzen mit abweichenden Chromosomenzahlen" (Zeitschr. f. Bot., Jahrg. 8, 1916, p. 417-531). — Winkler has succeeded in raising tetraploid individuals of both *Solanum lycopersicum* and *S. nigrum* by grafting, and has also been able to show that tetraploidy is on the whole transmissible, though irregularities may also occur. The fact that the tetraploid individuals now and again locally revert to the diploid type is, Winkler considers, due to reduction division in the somatic cells. The tetraploidy makes itself apparent in the increased size of the plants, and other peculiarities, which also distinguish the tetraploid *Oenothera* forms.

Strasburger (1904) writes in his summary: "Die Annahme liegt nah, dass übermässige Mutation die Schwächung der geschlechtlichen Potenz der Eualchimillen veranlasste und durch den Ausfall der Befruchtung die Anregung zur apogame Fortpflanzung gab" . . . and further: "Auch Diöcie hat in manchen Fällen den Anstoss zur Ausbildung apogamer Fortpflanzung gegeben, weil durch Trennung männlicher und weiblicher Individuen Befruchtungsmangel sich einstellte".

That fertilization is rendered difficult owing to marked mutation or to the separation of the sexes may be reasonable enough, but that this should be the cause of apogamous propagation in plants I cannot admit, and must reject the idea as a teleological explanation. "Befruchtungsmangel" is in itself an entirely negative principle. Overton (1904) follows the same line, with regard to *Thalictrum purpurascens*. as Strasburger; with this writer also I am unable to agree.

Moreover, we know of not a few monoecists which are apogamous, and dioecious plants which are not; for this reason also the theory that dioecism should be the cause of apogamy appears hardly reasonable.

It has occurred to me that there is one likely explanation of the occurrence both of apogamy and of the multiplication of chromosome numbers, which deserves consideration, but which does not appear ever to have been discussed (see, however, Ostenfeld's statements (1910) as to apogamous *Hieracia*) — to wit, that occasional hybridization might be the cause.

It will here be necessary to go further into the peculiarities of the chromosomes themselves. The most characteristic feature in these is, as we have already noted, their tendency to occur in pairs. Chromosomes are frequently found paired in the somatic cells, and continually in the gonokonts, the parent chromosomes exhibiting a mutual affinity. They are nowhere found in threes; neither in the nuclei of the endosperm, where 3x is nevertheless found, nor in somatic, hyperchromatic cells. Fours are likewise unknown, save where the chromosomes are in repeated division, as in the gonokont nuclei. Chromosomes once separated unite in pairs.

This evidently reveals some fundamental principle in the nature of chromosomes, and must be connected with the dualism to which every sporophyte owes its origin.

Generally speaking, only nearly related plants are capable of interfertilization and the production of progeny able to live on; it is therefore certain that only such gametes as harmonize physiologically — or better, perhaps, physiogenetically — can enter into the formation of a duality such as the sporophytic organism.

The gametophytic cell can rightly be considered as one harmonic whole, and we may further presume that only physiogenetically uniform nuclei — and especially their chromosomes — are able to affect each other. It will indeed doubtless be only reasonable to suppose that the greater or lesser degree of physiogenetic similarity or harmony will determine what common result is to arise from the union of two gametes.

I will now draw up a scheme showing the different degrees of physiogenetic likeness between gametes — and thus also between their chromosomes — endeavouring at the same time to ascertain what result we can expect in each case from the fusion of such gametes, basing my conclusions upon our knowledge as to the chromosomes in general, and of their occurrence in pairs in particular.

1. **Philozygoty**[1]). Two gametophyte organisms very markedly resembling each other are evidently found on comparing for instance two gametes from the same species of plant. Such homogamous cells may unite, their nuclei also unite, and the chromosomes appear in pairs, corresponding to the number of chromosomes — the pairing, however, being often noticeable first in the sporogenous cells, when a new gametophyte generation is in preparation. By philozygoty I mean the relation distinguishing such units as tend, by virtue of genetic, physiological or other similarity, to form, through fertilization, a common normal offspring, the sporophyte. Philozygoty must thus be said normally to characterize gametes belonging to one and the same systematic species.

The fact that self fertilization or inbreeding in many cases gives bad offspring or fails altogether must, I consider, be due to lack of mutual complementation, as a result of the over close relationship — a point to which I shal refer later on.

We may, however, also find that gametes derived from different species or forms have a harmonizing or corresponding

[1]) From *φιλο* in compounds = loving, and *ζῦγόν* a yoke.

"inner physiology" i. e. possess the qualities requisite for a pairing between their chromosomes, and thus the formation of a harmonic common product, a zygote with unimpaired vitality. Heterogamous organism may thus also be philozygotic. As in the case of the homogamous organisms, so also here the parent chromosomes are united in pairs — at any rate in the gonotokont nuclei, after which they again separate, the two sets of parent chromosomes being distributed on the daughter cell during reduction division.

Philozygoty thus denotes the highest degree of harmony between the sexual cells of two organisms.

2. **Pathozygoty**[1]). By this I mean to indicate the fact that two gametes may enter into the formation of a common zygote, which, however, owing to the less marked harmony between the constitution of the gametes, may often be only partly capable of developement, or not capable of developement in the normal manner.

This category will only include gametes derived from different species or races, i. e. heterogamous gametes.

When such heterogamous gametes unite, one of the following alternatives must take place:

A. Direct union of the chromosomes. If the parent chromosomes in accordance with the nature of chromosomes in affinity pair directly, we must presume that this will as a rule give rise to a zygote which may either be apogamous or normally sexed.

a. Apogamous forms. On the fusion of heterogametes, a zygote will be formed having the sum of the chromosome numbers in the gametes. When the chromosome numbers of the parent are different, the pairing will naturally be incomplete, but where the chromosome numbers are alike, a complete union of homologous chromosomes will take place. In both cases then, we have an apogamous zygote, and as the related species in a genus usually have the same chromosome number, x, the apogamous zygote will in most cases have 2x chromosomes. Apo-

[1]) From *παθεῖν* in the sense of endure or submit to. The same word being frequently used in the sense of suffering pain or damage, a better expression might possibly be found (Dyszygoti strikes me as an awkward combination). The Latin derivative pati is also used in the special sense for instance "concubitus pati" (Ovid, Ars Amandi 3, 666) and like expressions.

gamous species with normal chromosome number (compared with the remaining species of the genus) are also as a matter of fact found in nature — as for instance in the *Marsilia Drummondii* investigated by Strasburger (1907) which, like the remaining species examined, has $2x = 32$. From the synapsis stage, where the separation of the parent chromosomes is prepared, irregularities occur, so that reduction cannot take place, and the macrospore is formed by ordinary mitotic division. Some of the dioecious *Rumex* species examined by Roth (1907) are also believed to be partly apogamous, despite the fact that the chromosome number is not "doubled", and the species in question should thus agree with *Thalictrum purpurascens*, which, as Overton (1904) has shown, is capable both of normally sexed and apogamous reproduction. Should *Thalictrum purpurascens* really be produced by hybridization between two species, then Overton's results in this instance must probably be explained as due to a so complete union between the chromosomes that normal reduction may at times take place, at others not. The progeny of the *Thalictrum* in question should then be genotypically heterogeneous — a point which it would be most interesting to have investigated.

When difference in the chromosome numbers of the parent gametes renders complete pairing impossible, only a certain number of chromosomes uniting, the fertilization would probably also be imperfect, so that the propagating cells of the hybrid individual produced (somatic sexual products) would be rather of a gametic nature, and capable of further fertilization by a gamete with constitution able to give a more complete union of the chromosomes. There will thus be no question of apogamy — unless indeed the diploid sexual products of the individual were capable of independent developement despite the incomplete union of the chromosomes. This point will be further discussed later on.

b. Normally sexed forms. There is also the further possibility that the chromosomes meeting in the zygote may all, or some of them only, constitute a new chromosome set, when the zygote will thereafter represent something entirely new; a new species with a set of chromosomes rendered stable for the future, and normally sexed.

It seems to me as a matter of fact but natural to suppose, as I have also noticed in the foregoing, that the homologous or most closely harmonizing elements in the nuclei of the gametes

continually seek out each other in order to form harmonious unions, just as — to use a purely illustrative comparison — in chemistry, atoms of greatest mutual affinity constantly tend to unite.

B. Indirect chromosome union. Where a less marked harmony exists, we must then suppose that this will be visibly expressed by the fact that the chromosomes derived from the two gametes will not unite in pairs at all, but distribute themselves throughout the primary cell of the zygote, as if no dualistic relation of any kind existed. If the chromosomes are to find a partner, then each of the chromosomes in the zygote must divide, for thus indirectly to produce a union of chromosomes, and we must assume that this is realized in the hybrid zygotes which have any possibility at all of propagating — in accordance with what we know from experience as to the behaviour of pairs of chromosomes. The hybrid sporophyte thus produced will then have 4x chromosomes, taking the number for each of the parent gametes as x.

After this, either the chromosome pairs will have the power of further separating by reduction division, transmitting one set of the chromosomes from either parent to each of the gametes — in which case we have a new hybrid organism with the qualities of a pure species and "double" chromosome number; i. e. containing the sum of the chromosome numbers in the parent species. Or, if the power of reduction has been lost, but the power of continued existence otherwise retained, the result will be an apogamous species with 4x chromosomes.

Instances of species thus arising are, I believe fairly numerous in nature; we may doubtless assume that most of the species exhibiting "double" chromosome numbers are hybrids formed in this manner. It is perhaps not so easy to point out, in concrete instances, the parent species whence such "double" species are derived; the latter may even have altered since the time of their formation. Nevertheless, it is possible that we may succeed in doing so. By way of example, we might imagine *Atriplex patulum*, with its 18 chromosomes, as possibly derived from hybridization between *A. hastatum* and *A. littorale*, each of which has 9. The process of such formation would in such case be as schematically shown in Fig. 35.

With regard to the apogamous species, the chro-

mosome number here is, as we know, generally just twice that of the related normally sexed species, and as sexual sterility is itself characteristic of many hybrids, I consider it higly probable that apogamous species are derived from crossings between those normally sexed.

It is difficult to say whether the fact that the occurrence of apogamous species is relatively often associated with dioecism can also be explained by this means. On the one hand it may be urged that homozygotism is more easily maintained in

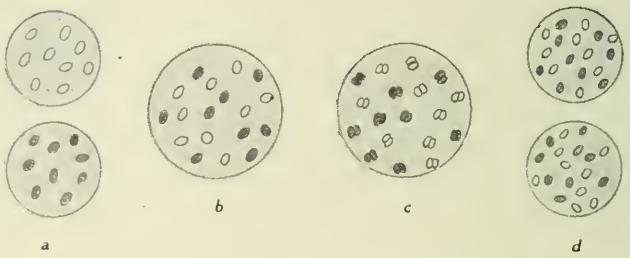


Fig. 35. Schematic view of occurrence of doubled chromosome numbers through hybridization. *a*, two heterogenous gametes each with 9 chromosomes. *b*, the hybrid zygote. *c*, indirect chromosome binding in the same. *d*, the hybrid gametes produced on reduction division, with 18 chromosomes.

monoecious than in dioecious species, where the possibility of absolute homozygoty is probably out of the question. The genotypic differences, and thus also the possibility of hybridization in species or varieties would naturally seem to be greatest in dioecious species. On the other hand it must be admitted that the dioecious species does demand a continual mingling of genotypes, whereas this is not necessarily the case in monoecists.

C. No chromosome union. Where the mutual harmony between two united gametes of heterogamous origin is so slight that their relation becomes almost disharmonious, the interaction between the two organisms will in all probability be of briefer duration and of a less intimate character. This might naturally result in the chromosomes of two gametes failing to unite either directly or indirectly. I believe I am justified in assuming that the chromosomes in sporophytes capable of sexual developement must continually act in pairs, and I must accordingly conclude that when neither direct nor indirect union of chromosomes takes place, then the organism is doomed, and can only exist as an

embryo — possibly with the power of cell division, as long as it is nourished by the mother tissues.

Experiments have convinced me that the pollination of a plant with pollen from another not of the same genus actually can result in developement of an embryo, and even in the formation of an apparently normal fruit, but that the embryo in such fruit is altogether incapable of independent existence. These experiments will be referred to later on.

3. **Misozygoty**[1]) Gametes of systematically widely differing organisms will as a rule altogether lack the power of uniting in a zygote. Not only is there too little similarity between the constitutions of such gametes; there is moreover no harmony between them, and in consequence, nothing calculated to produce the mutual affinity which is inseparable from all fertilization. The term misozygoty then, I use to designate this lack of mutual harmony: a reciprocal disinclination rendering fertilization impossible.

I must here repeat, that we cannot regard it as impossible for two distant organisms at times to be capable, by virtue of mutually harmonizing constitution, of uniting by cellular conjugation and forming something entirely new. We know of cases, where widely differing forms have united in symbiotic doubles. The symbiotic relation is marked by a more or less intimate union, in several instances of such a nature that the symbionts grow round or through each other, presenting the external appearance of one harmonious whole. It will suffice merely to mention the lichens and mycorrhiza as excellent examples of this. The latter moreover, further afford us an instance of one symbiont (the fungus) even growing intracellularly with the other (e. g. an orchid). I do not mean to suggest that the interval between this relation and a complete union should always be inconsiderable. It is often, in all probability, the very contrast which gives the symbiotic relation its value, each of the symbionts in question furnishing the other with the forms of nourishment which the latter is unable to obtain for itself. In my opinion, however, we can properly speaking find no just cause why a true cellular union between two organisms should not under certain circumstances take place, whereby a new organism could arise. Winkler's *Solanum* chimæras are of considerable interest, inasmuch as they

[1]) From *μισο* — in compounds indicating hate, aversion.

show that two organisms forming a common product can at times develope as a harmonious whole, despite the possession by each of the component of latent qualities which do not attain their developement. All epidermis formation, for instance, might be due to the one partner, albeit the other possessed a like power. This will serve to illustrate the conditions which we can imagine as existing in a zygote derived from two hèterogamous gametes. By suitable plasmatic and nuclear combinations and amputations, a harmonious zygote might be supposed to arise.

In the supposition as to the importance of hybridization, more especially as to a more or less intimate union between the chromosomes of the fused gametes — according as these are of more or less mutually harmonious constitution, — it will be understood that the very chromosome numbers which we find in nature might arise.

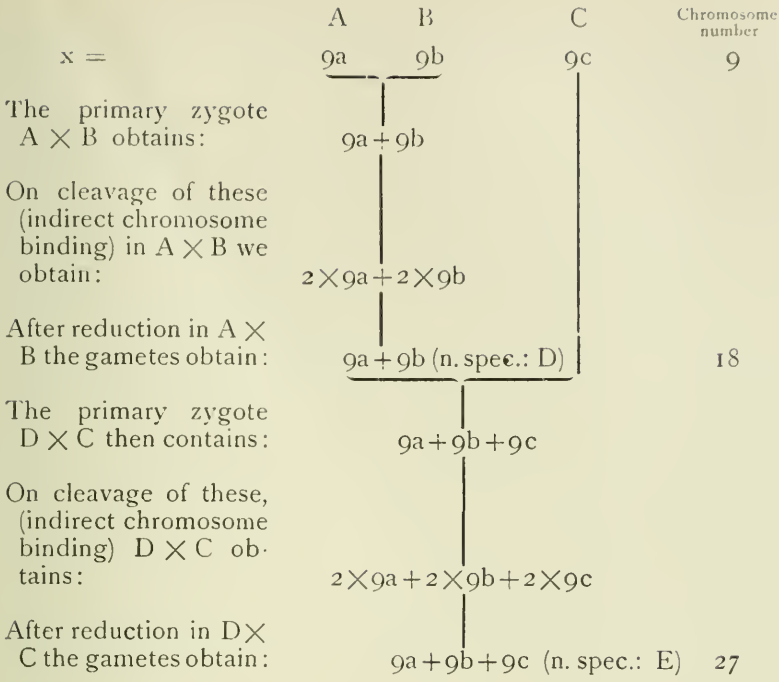
We may further consider the possibility that longer series of chromosome numbers within a single genus may be explained by the same means, taking, merely by way of example, *Chrysanthemum* as type. In *Chrysanthemum* we find, according to the investigations hitherto made, the chromosome numbers 9, 18, 27, 36 and 45, as we have already seen — and, as I have further shown, just such differential series are met with also in other spheres of relation.

As starting point we may take *Chrysanthemum* species with $x = 9$, this being here the cardinal number. Such species — it will here suffice to consider three of them — we may designate A, B and C; their chromosomes 9a, 9b and 9c respectively. On crossing these, we obtain results as shown schematically on page 203.

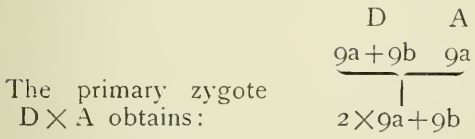
It will here be seen, that hybridization in the manner indicated results in the production of species with 9×1, 9×2, 9×3 chromosomes in the gametophyte, the chromosome numbers of the species paired being in each case added together in the offspring.

Naturally, again, other conditions will arise when the species thus produced are either intercrossed or paired with one of the parent species, since homologous chromosomes will then meet in the zygote, and direct union may take place.

In the case mentioned, we must suppose that there would



be formed a species, D, with 18 chromosomes (9a + 9b). On crossing these with A, we get the following:



How this zygote will behave, must depend upon circumstances; the constitution of the sporophyte may be more or less harmonious. It would be natural, however, that the 9a derived from D at any rate should unite directly with the homologous 9a from A. The 9b must either unite indirectly — i. e. if the sporophyte is to be normally capable of developement — or remain unpaired, when the sporophyte will be abnormal. Rosenberg's investigations (1909) with *Drosera* hybrids might be an instance of such a case. *Drosera rotundifolia* has x = 10, *D. longifolia* x = 20, and the hybrid 2x = 2 × 10 + 10, the 10 chromosomes from *D. rotundifolia* uniting with the 10 from *D. longifolia*, while the remaining 10 continue unpaired, so that a natural sexual further

developement cannot take place. We must from this suppose that 10 of *D. longifolia*'s chromosomes will be homologous with *D. rotundifolia*'s.

If my theory be correct, then we should occasionally find differences in the size and shape of the chromosomes, i. e. when a species had arisen by hybridization of two others with chromosomes differing in appearance. Also, chromosomes of one and the other type respectively should then be found in a proportion according with the characteristic cardinal number for the field of relation in question. A species A, with 6 long chromosomes, and a species B, with 6 short ones should on their union give rise to another with 6 long and 6 short.

Both shape and size of the chromosomes are, however, as a rule of considerably variable nature, and it is well known that absolute dimensions are difficult to apply. Some parts of the tissue have longer, others shorter chromosomes in the spindle, and probably also conditions of nourishment etc. affect their appearance. In the gonokonts, however, the chromosomes do appear in such a form that they may, when not too numerous, be studied and characterized as to shape. It would be most interesting to investigate the varying appearance of the chromosomes there, from the point of view already indicated. I will here cite a couple of examples which serve to bear out the correctness of my summation theory.

In *Spinacia oleracea* (Stomps 1910) the chromosomes are of different size, three long and three short. Stomps shows, for instance, in his Fig. A (p. 70) a fine nuclear plate from a syndiploid root cell, in which six pair of long and six pair of short may be observed. The sexual cells thus contain 3 long and 3 short chromosomes. Now 3, as I have shown, is the cardinal number for *Chenopodiaceæ*, and we have thus here an instance of chromosomes differing in appearance occurring in a proportion corresponding to the cardinal number, whereas a reduplication of an original three would necessarily give a different result.

In the zoological literature we find an instance eminently suited for comparison with the above

Chambers (1912) when investigating the American varieties of *Cyclops viridis* found the following chromosome figures, which are alike for male and female individuals:

Cyclops	viridis	12
»	var. Americanus	10
»	var. parcus	6
»	var. brevispinosus	4

The series of chromosome numbers, which thus has 2 as cardinal number, again affords an example of differential structure — albeit 8 is lacking here — and it is most interesting to note Chambers' statement that the 6 chromosomes in var. *parcus* were pairs of three different sizes.

Comparing this with the case of *Spinacia*, where the six chromosomes were 3 and 3 in two different sizes, we may doubtless take it that results stand in some relation to the respective cardinal numbers: in *Cyclops* 2, in *Spinacia* 3.

We have seen that series of chromosomes numbers such as we find in nature may be supposed to arise by hybridization, the chromosome numbers of the gametes thus united being generally added together in the gametes of the offspring.

But how, we naturally ask, could the chromosome numbers of the vegetable kingdom be as low as they are on the whole if they really were largely formed by addition? Evidently, it must be presumed that nature has some auto-regulative means of keeping the chromosome number down within suitable limits. There is of course some such limit for the number of chromosomes which a nucleus can, generally speaking, contain. Continued addition by repeated hybridization would soon give a theoretic chromosome number higher than any actually existing in nature. We must suppose then, that the supply of chromosomes in the non-dividing hybrid will under certain circumstances be simplified, but as to this we know nothing as yet.

It must also be noted, that the transition from misozygoty to philozygoty will naturally be gradual, since the categories given are based upon the supposition of more or less harmony or similarity between the gametes. And some pairs of organisms may therefore come to lie on the boundary line between two categories.

Finally, I would once more refer to the quotation from Strasburger (1910 p. 425) already given on p. 156 the last part

of which is associated with his idea as to reduplication of chromosome numbers in apogamous species. In the main, I cannot but agree with him here, albeit I cannot regard the "Vervielfältigung" of a previously existent number of chromosomes as phylogenetically important in itself, nor can I accept it as being the cause of apogamy; the supposition as to hybridization, on the other hand, appears to me to afford a natural explanation.

I must in this connection maintain, that the various haploid chromosome numbers occurring in the species of a genus are not correlated on the principle of a, 2a, 4a, 8a, etc., as chromosome reduplication would involve, but on that of a, 2a, 3a, 4a, etc.; i. e. in arithmetical, not geometrical progression. That powers of 2 are particularly frequent in chromosome numbers is, moreover, but natural, since presumably original species with the same chromosome number are especially adapted to the production, by hybridization, of new species, which would thus acquire a "double" number.

In estimating the degree of relationship between the chromosome numbers of higher plants, we should accordingly attach chief importance to the ascertaining of the cardinal number. The presence of the cardinal number 2, on the other hand, can be due to the "reduplication" mentioned, and thus tells us nothing as to the possible relation between the two chromosome numbers in question. When, however, we find powers of 2 characterizing several chromosome numbers the relationship of which it is desired to estimate, so that 2n is a common factor, there is more reason to regard the 2-factor as indicative of such relationship — and higher the power of 2, the more likely will it be.

CHAPTER 5.

HETEROCHROMOSOMES.

The occurrence of "heterochromosomes" or "accessory chromosomes" differing from the normal in various respects is more particularly known in connection with animal organisms. Henking (1891) was the first to find a special chromosome of this kind in some of the spermatozoa of *Pyrrhocoris apterus*. Mc Clung (1902) hit upon the idea that such chromosomes might be sex-determinative; i. e. that the germ containing a heterochromosome

would be of different sex to that containing none; it remained for Wilson (1905) to prove that this was, in certain instances, actually the case. Numerous zoologists, Sutton, Stevens, Morgans and others, have since gone farther into the great question as to the importance of heterochromosomes, with the result that the matter now appears less simple than the first investigations seemed to suggest.

As an example of the occurrence of heterochromosomes, I may note the process of reduction division in *Gryllus domesticus*, as investigated by Gutherz (1907). On the division of the spermatogonia, a larger chromosome is observed, differently colourable than the remainder, and placed apart. This heterochromosome divides together with the rest, when the formation of spermatocytes takes place, each spermatocyte obtaining its own heterochromosome. Where a spermatocyte splits up into tetrads, only one of the præspermatides obtains the heterochromosome, which moves undivided to one of the poles. At the subsequent division, which gives spermatides, the heterochromosome is again divided, so that two of the spermatides are furnished with heterochromosomes, the others lacking these. — Half the male sexual cells will then be different from the other half, which should give a difference in the sex of the offspring.

In other organisms, however, modifications have been found which to a certain extent impair our confidence in the general power of the heterochromosomes to determine sex.

Boveri (1911) and Schleip (1912) for instance, has found the following cytological conditions in *Angiostomum nigrovenosum*, where a hermaphrodite generation alternates with a distinctly sexed. The hermaphrodite generation has 12 chromosomes in the ovogonia, viz: 4 small and 8 larger. The primary and secondary ovocytes obtain 12 and 6 chromosomes respectively. The secondary ovocytes then again divide normally, so that the female pronucleus always has 6 chromosomes. — In the spermatocytes of the hermaphrodite generation of the first order there appear first 1, then 2 heterochromosomes ("monochromosomes"), making in all 10 + 2 (the 2 being heterochromosomes). The first spermatogeneous division, in which reduction takes place, results in spermatocytes of the second order, with 5 + 2 (heterochr.) the 10 undergoing reduction, while the 2 are equally divided. During the second spermatogeneous division, again, 5 normal chromo-

somes move to either pole after equal division, and of the two heterochromosomes, one goes to each spermatide. — It is thus seen, that while the first spermatogeneous division involves reduction of the normal chromosomes, the number of heterochromosomes is not reduced until the last division. The spermatides also will be different, only half of them retaining the heterochromosome, which is discarded from the others and left to perish. Here then, the difference in the chromosomes of the separate sexual cells is not arrived in the usual manner; is not an original process, and this suggests that the number of chromosomes is not of any fundamental importance for the determination of sex. — The embryos produced on fertilization will then naturally have 11 and 12 chromosomes respectively, and may be supposed to be male and female accordingly.

Again, the Hemiptera *Nezara hilaris* has (Wilson 1905) both in the spermatozoa and in the ova, 2 equally large heterochromosomes, and yet we find both male and female individuals. Wilson's hypothesis, that it is not the difference in the chromosome numbers as such, but physiological or functional factors which determine sex, therefore seems more likely to be correct

The occasional autoregulative discarding of chromosomes during reduction division, as referred to above, is a point of great interest, being evidently associated with, and serving to explain, the fact that somatic tissue is not always found to have a chromosome number in accordance with the characteristic figure for the sexual cells of the species; this I have mentioned in the foregoing. This discarding of chromosomes and chromatic matter is known by the name of "chromosome diminution" and has been observed in various organisms (Boveri 1887, Bonnevie 1901, Kahle 1908, Buchner 1915). — In *Ascaris megalocephala* (Buchner) and *Miastor* (Kahle) for instance, we find chromatin and chromosomes discarded already during the first cleavages of the embryo. The first division, however, proceeds normally, and the cell which later forms the mother tissue for the sexual cells retains a full set of chromosomes, whereas the somatic cells have less than 2x.

The number of those who still adhere to the idea as to existence of sex-determinative chromosomes is steadily decreasing, and from our present standpoint we may maintain that it is not the heterochromosome which is the primus motor in sexual

differentiation, though it may, in several cases, more particularly among insects, be associated with one or the other sex, chiefly the female. It is thus rather a question of correlation between the appearance of the chromosome set and physiological or other conditions which determine the sex of the organism (The "Index hypothesis" of Häcker).

Turning now to the vegetable kingdom, we here find further proof that the sex does not depend upon presence or absence of any heterochromosome; in vegetable organisms, there is on the whole nothing corresponding to the mentioned conditions.

The case cited by Cardiff (1906) of the *Polygalacea Salomonia biflora*, where an unpaired chromosome was observed, cannot be ascribed to the same category, as the plant with which the writer in question is concerned is not dioecious at all. Similarly inapplicable to the present question is the remarkable case of *Empetrum* (Samuelson 1913) where the male and female gonokonts were found to have a widely differing number of chromosomes[1]).

All other dioecious plants hitherto investigated have the numbers of chromosomes in the two sexes corresponding, and a not inconsiderable number of dioecious plants have been investigated especially with a view to the possible discovery of chromosomatic differences. *Hydrocharis morsus ranæ*, *Bryonia dioica*, *Lychnis dioica*. *Mercurialis perennis*, *Sagittaria Montevidensis*. *Cucurbita pepo*, *Cannabis sativa*, *Humulus lupulus*, *H. Japonicus*. *Urtica dioica* and various dioecists have thus been cytologically investigated, without any such differences having been discovered.

That sexual difference may nevertheless exist in the nuclei — even though it may not be immediately perceptible under the microscope — is shown by Burgeff's interesting *Mucoraceae*-mixochimæras. Burgeff's experiment (1913) seems, as a matter of fact, to show that each nucleus in the organism examined constantly belongs to the one of the two sexes. — Bakeslee, in his studies on the sexuality of *Mucoraceae*, found that the sporangium from a zygospore or *Phycomyces nitens* contained spores of three sexually different types, viz: positive, negative and neu-

[1]) Later note. According to verbal communication from the writer in question, the discrepancy between the number of female and that of the male chromosomes is doubtless due to an erroneous count owing to unsatisfactory material.
Ö. W.

tral, only the mycelia of the two first being capable of interfusion. Following on this discovery, Burgeff advanced the hypothesis that the nuclei of the mycelia were the really positive and negatively disposed, and that neutral mycelia were only such as contained both positive and negative nuclei. As the spores of *Phycomyces* contain one or more nuclei, the spore will contain now exclusively positive, now only negative, nuclei, or again a mixture of both, and the same will be the case with the mycelium, which thus becomes either "homokaryotic" or „heterokaryotic". Burgeff demonstrated, by experiment, that this actually was the case. The tip of a young sporangiophore was inserted into the basal part of another sporangiophore of the opposite sex, which had been cut through; by pressure on the latter, the tip of the one inserted was burst, so that a quantity of cytoplasm with nuclei was introduced through the hole in the cell wall. After the operation, the inserted sporangiophore was induced to form sporangium, and in this all three kinds of spores were also found: positive, negative and neutral, as was proved by continued cultivation of the spores.

I must nevertheless here point out that the chromosomatic conditions in *Mucoraceæ* cannot be directly compared with those of the higher plants, and that the alternations of generations itself is in these too little known. The mycelia are constantly gametophytic, and the diploid stage is believed to be altogether of extremely short duration — restricted to the elder zygote (Moreau 1913) where a reduction should then take place. Whether the gones (nuclei) in course of the reduction develope a "plus" or a "minus" sign is still entirely unknown.

Apart from such peculiar conditions as those here mentioned, however, we can yet find instances in the vegetable kingdom where a kind of heterochromosomes also can occur.

I shall in the following have occasion to make particular mention of *Callitriche verna*, in which I have been able to observe certain cytological phenomena bearing on this point. — The plants from which my material was procured grew in a cement basin in the Botanical Garden of the University at Copenhagen, and appeared to thrive extremely well. Numerous male and female flowers and fruit at various stages of developement could be observed throughout the summer. I collected and fixed

material twice during the period of vegetation, and in both cases the results obtained were the same.

The earliest stages of developement in the pollen mother cells were poorly represented in my material, but as far as could be seen, there was no deviation from the normal. This evidently did not set in until reduction division, more especially during the

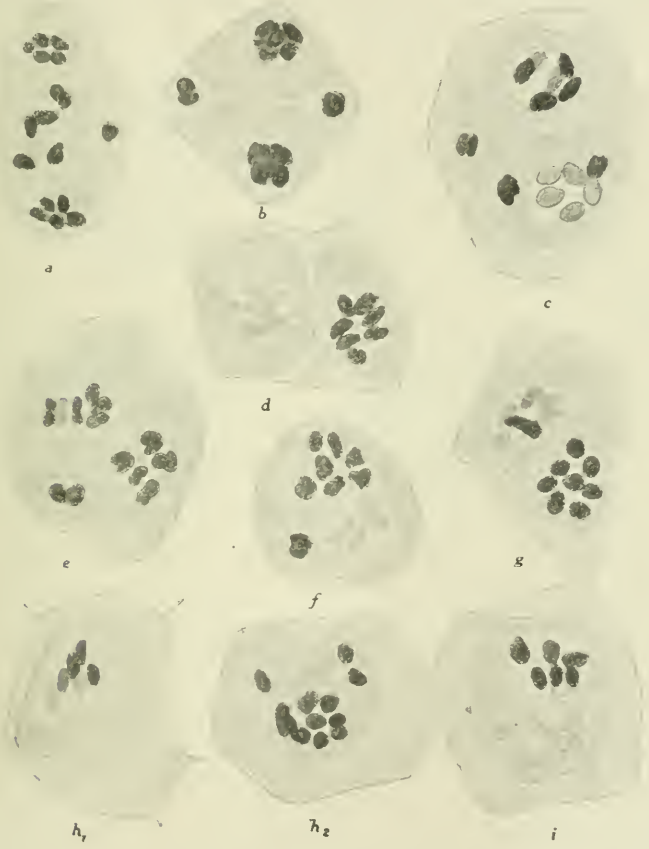


Fig. 36. *Callitriche verna*. Pollen mother cells. Reduction division with formation of "heterochromosomes". See explanation in text.

anaphase of the heterotypical division. The chromosomes, which were present to the number of 16 (= 2x) are distributed somewhat irregularly throughout the spindle (Fig. 36*a*); some have already reached their poles, while others have hardly yet left the original plane of the nuclear plate — or are on their way towards the poles. It is here particularly noticeable, that one or two chromosomes remain lying in the extreme periphery of the nuclear

plate, apparently beyond the region of the spindle itself. These — there are as a matter of fact generally two — are thrust out from the sphere of action of the spindle, and left to their fate. The two chromosomes are undoubtedly "reduced", but they do not, as far as I have been able to discover, represent a correlated pair of chromosomes from the diakinesis and the metaphase, as their position in the nuclear plate (the symmetrical plane) is not face to face, but as a rule diametrically opposed. There are thus only 7 chromosomes which migrate to each of the daughter nuclei, and these last are separated by a wall, albeit a very thin one, in contrast to what we usually find in dicotyledonous plants. A frequently occurring state of things is shown in Fig. 36*b*, illustrating the telophase; the two ejected, diametrically opposed chromosomes are still in the equator, and it will further be seen that each of them is distinctly divided into two. The two chromosomes have a very slight quantity of cytoplasm at their disposal, and are seen to be surrounded by a fine line, evidently indicating that they behave as relatively independent nuclei.

I should here at once point out that the process here concerned does not take place with absolute accuracy. I have thus quite undoubtedly observed 8, and even 9 chromosomes at the one pole, with a correspondingly smaller number at the other. Despite this, the heterochromosomes were visible as independent elements. On one occasion I could discern three of them (Fig. 36*c*).

The heterochromosomes — as I will call them for the sake of convenience, since they correspond in various respects to those known from zoology — are now seen to be constantly individualized; not until the metaphase of the homoeotypical division does any distinct alternation take place, the splitting then becoming more conspicuous, — and appearing, now as a true separation, now giving the chromosomes the shape, approximately, of a dumbbell. The same splitting takes place in the normal chromosomes, which thereby prepare for the homoeotypical division, and in the metaphase of this division we often find that the normal chromosomes have completed the separation, so that more than 7 chromosomes are observable (Fig. 36 *d*, *e*, *f*, *g*, *h*, *i*).

In the course of the homoeotypical division, the heterochromosomes are now either separated entirely, whereby 4 small nuclei arise, or, more frequently, the splitting products remain

lying together, so that only two nuclei are visible, each of them containing a distinctly divided chromosome.

After the homoeotypical division, we have then a colony somewhat variable in appearance, arising from the spore mother cell. As a rule, a hexad is formed by 4 large and two small nuclei, probably with 7 chromosomes in each of the large ones, but only one double in each of the two small (Fig. 37, *a*, *b*, *c*, *d*). At times — i. e. when the homoeotypical division has been com-

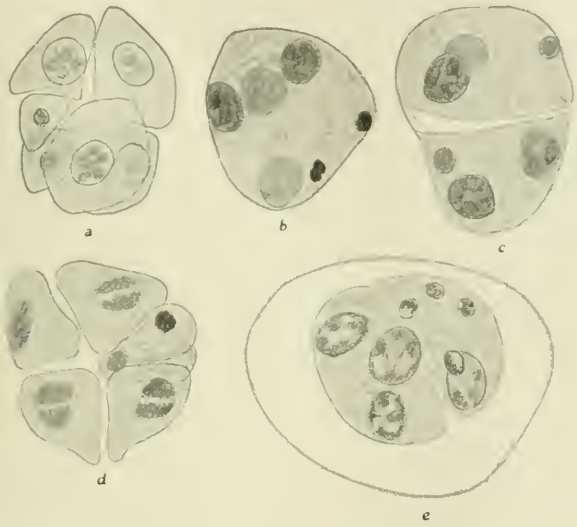


Fig 37. *Callitriche verna*. Pollen formation. Hexads and octads. See explanation in text.

pleted also in the case of the heterochromosomes — 4 large and 4 small nuclei may be seen (Fig. 37 *e*). The large ones are throughout separated one from another, each in its own cytoplasm area; the small ones, on the other hand, do not always form separate cells, but may often enter into the cytoplasm area of one of the large nuclei, which thus contains one large and one small nucleus. — In other cases, the two small nuclei together cut off a piece of cytoplasm around them. — As the final result of all this, a number of pollen grains are formed in the pollen sacs, varying greatly in size according as they contain more or fewer chromosomes. It is interesting to note that even the smallest cells, only containing one nucleus with a single chromosome, are able in some degree to maintain their position as pollen grains, forming a yellow exine of similar composition to that of the larger

pollen. This is important from a theoretical point of view, as it appears to show that a single one of the many chromosomes in a nucleus — i. e. a fraction of the chromatic substance which it contains — is capable of producing what is in fact a normal grain of pollen: a gametophyte. Another point worth mentioning

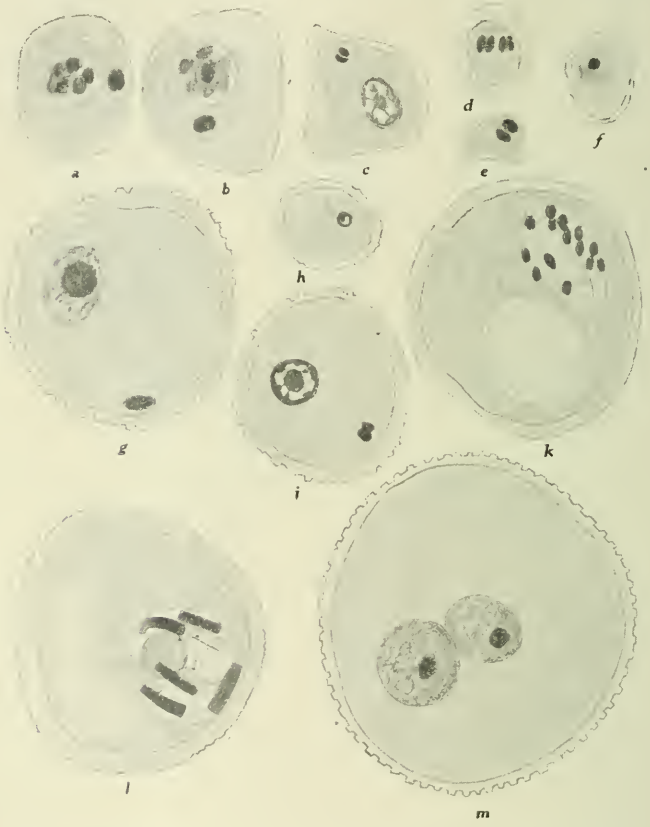


Fig. 38. *Callitriche verna.* Pollen formation. Pollen grains of varying size and with varying chromosome number. Vagrant chromosomes. See explanation in text.

is the fact that it is generally split, but not homoeotypically divided chromosomes which form the small nuclei and pollen grains. The nuclei, and to a certain extent also the chromosomes, can be very nicely observed in the pollen grains themselves (Fig. 38, *a*—*m*) even when the latter are mature. The smallest pollen grains contain only one split chromosome, while few contain two such; others have but one large nucleus, but in

others we may also observe a dwarf nucleus with a split chromosome situate somewhere in the cytoplasm. — As a rule, of course, it is impossible to determine how many chromosomes each pollen grain contains, when there are more than one or two, but that there can, under certain circumstances, be 8 of them in the nucleus of the pollen grain, will be very distinctly seen from Fig. 38 *l*, showing the cell of the pollen grain during division. Such a pollen grain will then either have been produced by normal tetrad division, or by the subsequent reuniting of one of the heterochromosomes with the main nucleus. The former explanation seems to me the more likely, especially as I believe to have observed some few normal heterotypical divisions. From the nature of the case, however, it is difficult to make sure that it is not due to faulty microtomy when the picture produced does not reveal any heterochromosomes. That union of the two nuclei can, however, take place, I consider probable. I would here merely call to mind Němec's experimental investigations (e. g. 1902) which have already been referred to under another head.

We see then, that normal pollen grains — i. e. with 8 chromosomes — can occur, and such must probably also be regarded as necessary if a normal germ is to be produced through fertilization. It would nevertheless be interesting to consider further the possibility that isolated small nuclei can take part in fertilization, so as to produce germs with the normal number of chromosomes by this means. That this should be so must at the outset appear doubtful. As already mentioned, the plants under consideration had numerous fruits, and these contained an apparently normal germ. As my fixed material was not suited to cytological investigation of fertilization, it must be left for future research to determine whether this fact can afford any explanation of the divergent conditions[1]).

Pollen formation in *Callitriche verna* does not take place in a certain definite manner, but is subject to several variations; it is therefore hardly to be doubted that we have here to deal with abnormal conditions, whether arising either from purely local conditions, or — as I am inclined to suppose, from the nature of

[1]) Later note. After reading the Danish edition of this paper, Rosenberg by letter proposes apogamy in *Callitriche* as a possibility. I consider it very probable that this may be the right solution.

the plant itself. Even though the formation of hexads of pollen grains may be said to be the rule with *Callitriche verna* — as my material suggests — it would nevertheless on the whole seem that the phenomenon is rather theratological. That the heterochromosomes, in any case, have here nothing whatever to do with the difference of sex, is obvious, if only from the fact that the pollen grains in the hexads have generally all fewer than 8 chromosomes, viz: 7 (the 4 large pollen grains) and 1 split (the two small).

The phenomenon observed in the case of *Callitriche verna* is not unique; irregularities in pollen formation similar to those mentioned are known from several plants. As far back as the time of Hofmeister it was noticed that "Sonderkerne" might arise during pollen formation, and from later investigations, *Hemerocallis fulva* has become a classic example of this peculiarity. Strasburger (1882) and afterwards Juel (1897) found, in the case of this plant, that a relative independence was noticeable in one or more of the "zurückgebliebene" chromosomes, these being left over at the heterotypical division, and forming small nuclei. Similarly also, Tangl (1882) and Fullmer (1899) have observed irregularities in the same plant; these last two writers, however, wrongly believe the smaller nuclei to have arisen through indirect division of one of the tetrad nuclei. Wille (1886) mentions 17 species in which an abnormal number of pollen grains is formed from the pollen mother cell, sometimes less than 4, sometimes 5, 6 or more. This writer likewise erroneously considers the small cells as division products of one of the original 4 in the tetrad, but no detailed investigations as to the chromosomes have here been made.

In *Syringa*, again, as investigated by Juel (1901), pollen irregularities occur, especially in *S. Rothomagensis*, which is believed to be a hybrid between *S. vulgaris* and *S. Persica*. The irregularities are of various character, but Juel notes, inter alia, that in course of nuclear division, chromosomes are ejected to the cytoplasm, and that they surround themselves with a wall. Juel is of opinion that these phenomena are mainly due to the hybrid nature of the plant, and there is no doubt that this also is the case.

The researches of De Lary de Latour (1908) with *Agave attenuata* are of particular interest in connection with the facts

noted in the case of *Callitriche*. Here also the writer in question finds that small nuclei are formed in the equatorial plane, and he presumes that they are destroyed, or fuse together with the principal nuclei: "et l'on ne les trouve que très rarement dans les grains de pollen formés" (p. 835). This then is one of the few plants for which I have found any record as to the continued existence of the heterochromosomes in the pollen grain. As far as I can understand the writer's meaning, however, they seem to disappear in a short time.

In a pteridophyte also, abnormities in spore formation have been noted, viz: in the *Polypodium vulgare* var. *elegantissimum* investigated by Farmer and Digby in 1910. The variety, which was found growing wild, differs from the main species in having the pinnæ markedly lobed; some few pinnæ of the usual form are, however, often observed. It was found, that this variety — in contrast to *P. vulgare* — exhibited on tetrad division a number of chromosomes which had placed themselves irregularly in relation to the spindle, being, so to speak, beyond the general sphere of control under which division proceeded. — The writers quoted opine, that this phenomenon is connected with the restricted power of spore formation in the variety, and they state that it has a slightly higher chromosome number than the main species, the latter having "90 at the outside" and the variety referred to "at least 90".

In *Oenothera lata* × *gigas* (the *lata* type) again, Geerts (1911) has found some interesting features, corresponding, moreover, on the whole to the results published by Rosenberg with regard to *Drosera* hybrids. The parent plants have $x = 7$ and $x = 14$ respectively, and the hybrid then obtains $2x = 21$. In reduction division, a partial pairing takes place, — precisely as in the *Drosera* hybrid, the 7 chromosomes of *O. lata* being united with 7 of those in *O. gigas*, the remaining 7 from the latter continuing unpaired. The paired chromosomes distribute themselves normally, and the separate chromosomes likewise pass, on the heterotypical division, to either pole, generally 3 and 4 to either side; on the second division, however, the unpaired ones are often broken up into small pieces, which either group themselves irregularly, or are cast out entirely, or enter into the young pollen grains (or the embryo sac respectively) in the form of dwarf nuclei, which later disappear. — As in the case of *Callitriche*

then, so also here dwarf nuclei have been observed in the cytoplasm of the pollen grains.

Mention should also be made of Nawaschin's investigations (1911) regarding phenomena in *Tradescantia Virginica*, which strongly resemble those I have shown to exist in *Callitriche*; here also, in the course of the heterotypical division, a chromosome moves out into the equator, where it may be seen lying "in a vacuole". On tetrad division, however, according to Nawaschin, 4 cells are produced, viz: two containing 11 chromosomes, and 2 with 11 + 1. If the chromosome distribution really does proceed regularly here, as stated, we have then a phenomenon markedly resembling the dimorphism well known in the spermatides of the *Arthropoda*. The writer in question himself emphasizes this fact, but is inclined to doubt whether there is any real homology, and suggests the possibility that the irregularities found may be due to unfavourable weather conditions.

Finally, I will merely call to mind Rosenberg's well-known and most interesting works on the *Drosera* hybrids (e. g. 1909 *c*) and on apogamous *Rosa* species (1909 *b*) where small nuclei are likewise formed, and chromosomes are ejected during the tetrad division, so that isolated chromosomes may even at times be observed in the pollen grains after the first generative division therein. — These investigations will be further treated in a later chapter.

Summing up the results of the above as to the behaviour of heterochromosomes in plant organisms, we find that a special kind of these elements is really found in certain plants. Several of these are, however, forms known to have originated from hybridization between "good" species — and others, such as the *Polypodium* referred to, may possibly be of the same nature. It must nevertheless be admitted that the irregular tetrad division is also met with in apparently "good" species, and indeed, that the heterochromosomes in these very cases occur with the greatest regularity, which renders the question less easy of comprehension. — It does not appear to me likely, as Nawaschin suggests, that weather or similar conditions should give rise to a phenomenon so markedly regulated as this. I am inclined to believe that we shall in time perhaps be able to distinguish more finely

than at present, even where we now believe a species to be "good", as for instance *Callitriche verna*. I could imagine that genotypic differences might be present within one and the same species, causing the extreme forms, on intercrossing, to act very much as do entirely different species — i. e. produce offspring in which the parent chromosomes are not normally paired, which again would involve the further consequence that the formation of sexual cells must proceed abnormally or even not take place at all.

There is no suggestion made that the plants I have here referred to — save for the hybrids and apogamous species — were incapable of sexual propagation, nor had I the impression that my *Callitriche* plants were abnormal in this respect[1]). Unfortunately, I did not collect fruits of the same individuals which I subjected to cytological investigation. It would have been interesting to determine whether they were capable of propagation or not. — As I have said, the normal tetrads on pollen formation were not numerous, — if any such indeed were present — and if the *Callitriche* fruits had arisen through fertilization and were capable of germination, there would then hardly be any other possible explanation than that the dwarf nuclei referred to at times unite with the main nucleus in a pollen grain. That the heterochromosome was "reduced", but had not undergone the homoeotypical division, I cannot regard as any obstacle to the formation of a pollen grain nucleus normal in itself. On the other hand the possibility that a normal fertilization, i. e. such as leads to the formation of normal offspring, should be able to take place with an incompletely equipped pollen grain, must, I consider, be excluded.

The frequent appearance of plant heterochromosomes in sexually abnormal forms is, it seems to me, a further indication of the correctness of my view as to the importance of chromosome pairing. Future researches must decide whether the "good" species in which heterochromosomes are found should not really be classed as hybrids.

[1]) Later note. The possibility that the development of fruits was due to apogamy in *Callitriche* must, however, not be precluded. The apogamy might than have come into existence by hybridization of different forms of the species (see note on page 215).

CHAPTER 6.

THE PERSISTENCY OF CHROMOSOMES, AND THEIR HEREDITARY SIGNIFICANCE.

The number of chromosomes in the gones is constant for each species, individualized chromosomes entering in a certain definite number into the nucleus of the gones. This fact in itself makes it reasonable to suppose that the chromosomes are not casually occuring elements, but on the contrary, of a highly persistent nature, and closely connected with the organism and the whole constitution of the same. True, we cannot always discern their presence in resting nuclei, but as soon as the nucleus undergoes division, they again appear as highly chromophile bodies. If they were constantly individualized in the cell while the latter was at rest, i. e. not in process of division, then they must there have been of a different chemical organisation, possibly also possessing other physical qualities than during the division period of the nuclei (see Lundegårdh 1913 a and b). Moreover, in various organisms, the chromosomes can be discerned even during the resting period of the nuclei, where they appear as small individualized bodies: not, perhaps, with the usual form and size characteristic of chromosomes generally, but extremely often in the very number theoretically to be expected. There can therefore be no doubt that the chromosomes are of such a nature as, once formed, to persist within the nucleus until the death of the latter (see also Tischler 1915).

What more especially interests us here is the fact that the chromosomes, not only in the gonokonts, but also in somatic tissue, in the form of prochromosomes, may at times be seen lying in pairs. Rosenberg, who has largely contributed to the results attained in the investigations as to the persistency of chromosomes (e. g. 1904) is of opinion that the chromosomes in the somatic cells are also frequently paired (1909) and the same was found by Cannon (1903) in the case of *Pisum sativum* during the vegetative divisions of the anthers, leading to a separation of the tissue of the sporangium wall, and the sporogeneous tissue. Laibach also (1907) makes mention of paired chromosomes in resting somatic nuclei of *Sisymbrium strictissimum*, and states with regard to this (p. 200) "Nicht alle Körner lagen paarweise,

doch häufig konnte man drei bis vier Paare in einem Kern erkennen". Overton, again (1905) has observed similar conditions.

We can, then, I consider, conclude from such concrete observations, that in normal plants, there is always a certain interaction taking place between the parent chromosomes in the somatic cells. In accordance with what I have previously mentioned, I must regard it as certain that such interplay, which is undoubtedly an indication of dualism in each individual somatic cell, is necessary to all normal reproduction. The pairing need not perhaps always be visible, just as the chromosomes themselves are not always so, but the mutual affinity between the parent chromosomes in pairs is, I opine, present in normal plants. Where such affinity is lacking, as frequently for instance in the case of hybrid forms, the situation described on p. 199 arises, and may lead to reduplication of chromosomes and the formation of apogamous species.

The question as to the hereditarily transmissible qualities of the nucleus, and especially of the chromosomes, is a subject which has frequently been discussed, and widely divergent views are still maintained as to which parts of the sexual cells possess the transmitting power. Mysticism apart, it must be presumed that the predisposition to the later developed qualities of an organism will be localized either in the cytoplasm and the plastides etc. which it contains, or in the nucleus — if not in both. Bearing in mind that the nuclei, and particularly the chromosomes, are organs which in the phylogenetically lowest forms are less individualized than in more highly developed organisms, — a point which I have endeavoured to prove in a preceding chapter — this in itself must lead us to conclude that cytoplasm and nucleus are not themselves elements of so widely different nature that there can be any question of "either-or"; the choice of an alternative is here nullified by the close relation between both organs. It is possible, however, that the nucleus, and especially the chromosomes, may be more particularly the bearers of certain qualities, which are not localized in the cytoplasm or the reverse, and we have already proofs of this in the results furnished by experimental research in the field of heredity. I will here mention some investigations and experiments which I have myself carried out in this respect, the results of which entirely agree with those previously arrived at by other writers.

First, as regards the cytological investigations, I collected material of *Chelidonium majus* and its variety *laciniatum* with markedly divided leaves, also of *Sambucus nigra* and its variety *linearis*, which has extremely finely divided leaves, and *aurea* with speckled leaves. My object in selecting these was chiefly to ascertain, if possible, whether any differences could be shown to exist between the numbers or shapes of the chromosomes in the main species of these plants and their varieties. The cytological data, concerning *Chelidonium*, I have already quoted on p. 183 and it now only remains to repeat that no absolute difference could be discerned in any instance. I mentioned, at the same time, that the separation in the chromosomes of *Chelidonium*, the formation of *Sarcina*-like chromosomes, was perhaps more conspicuous in var. *laciniatum*. How far this may have been due to other than accidental causes, however, I am unable to say, and it would require a large amount of cytological material to decide the point.

With regard to *Sambucus*, I could discern no difference at all (see p. 190). The main species, and the var. *aurea* and *linearis* were apparently entirely alike.

It should in this connection be noted, that while *Chelidonium majus* var. *laciniatum* is stated as being an hereditarily fixed form, some doubt exists as to whether the like applies to the *Sambucus nigra* varieties. As a contribution towards the solution of this problem, I gathered and sowed seeds of var. *linearis* and the mottled variety, *aurea*. The seeds collected were probably produced by alien pollination, which proved an obstacle to the decision of the question. My experiments with selfpollination of *Sambucus* — they include, it is true, only the yellow-mottled variety mentioned — have, however, convinced me that this is unsuccessful, as the clusters which I had isolated for the purpose produced no fruits at all, whereas the experimental plants otherwise bore numerous berries with well-developed embryos. The sowing gave good seedlings of both varieties, which are now three years old, but none of the plants presents the impression of having departed from the main species, neither the yellow mottling nor the fine division of the leaves being apparent in any instance. I am of course aware that this experiment of mine does not furnish much in the shape of positive results, as it is even likely perhaps, that F_1, if derived from pollination of the eggs of the

variety with "normal" pollen, may be altogether dominated as regards the qualities in question by its normal father, as other experiments have shown is generally the case. Strictly speaking, therefore the results here merely show that the qualities which characterize the varieties as distinct from the main species, i. e. the finely divided shape, or yellow edges, of the leaves respectively, do not dominate in F_1 — when crossed with a possibly normal plant.

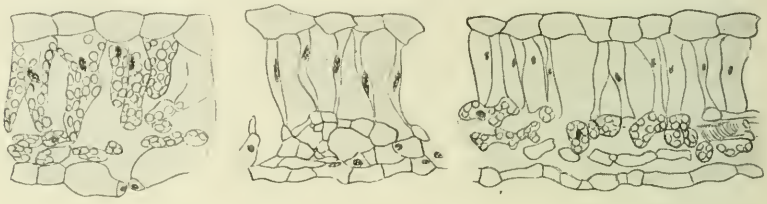
With regard to the chromosomes, however, we find that it is hardly possible to discern morphological differences in the nuclei of these divergent forms, and the same result has been arrived at by other investigators dealing with different plants. White (1913) has for instance studied the chromosomes in a twisted *Nicotiana* plant in order to ascertain whether it differed cytologically from the normal race, but he was likewise unable to discern any difference. The number of chromosomes, and all other features, were alike in both. Stomps (1910) has investigated in the same way several races of *Spinacia oleracea*, I myself white-mottled and normal races of *Humulus Japonicus* (1914) etc. — always without any positive result.

This is not surprising. When we remember that we are altogether unable even to characterize the chromosomes of the different plant families — that we cannot so much as distinguish between animal and vegetable chromosomes, — how can we then expect to find so slight a morphological deviation indicated by the particular shape or number of the chromosomes? The hereditary differences are not themselves visible, it is not the morphological differences in the chromosomes, but variations of a far more delicate nature, which give rise to the external morphology — and further, the differences may, at any rate at times, be due to cytoplasmic peculiarities.

I now pass on to describe the experiments I have made in respect of the last-named feature.

As we know, there are several races of the Japanese hop, *Humulus Japonicus*, an annual plant, which, like *H. lupulus*, is dioecious. The handsome white-mottled variety, var. *albomaculata*, is well known as an ornamental plant, and it is this sort which I shall more particularly have occasion to mention. *Humulus Japonicus* is distinguished by a growth which for an annual plant must be called enormous, as long as it is cultivated under favour-

able conditions, with plenty of warmth and richly manured soil. Towards autumn, the plants in a growth house may often have reached a height of 5-6 metres, and a considerable extent. Fruits both of the normal green race and of the mottled variety can be procured in the ordinary course of trade; the latter sort is characterized by having the leaves more or less spotted — as if sprayed with lime-wash. The light spots on the leaves, which are often so large that the leaves themselves might rather be called "white with green spots" are not always quite chalk-white in colour; various intermediate tones occur, as a rule three or four such, according as more or fewer layers of the parenchyma are free from chlorophyll. Fig. 39 shows transverse sections of



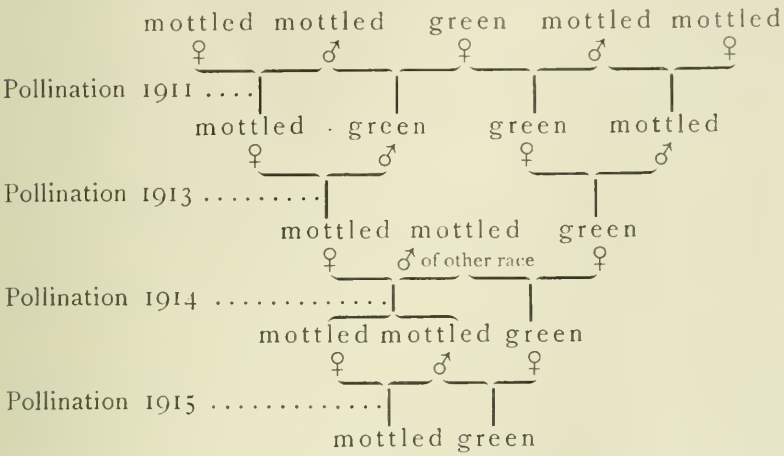
a *b* *c*

Fig. 39. *Humulus Japonicus* var. *albomaculata*. Transverse sections of leaves *a*, normal green part of leaf. *b*, part entirely free from chlorophyll. *c*, portion with parenchyma partly containing chlorophyll.

leaves, which at the parts in question were either normally green (*a*) or quite free from chlorophyll (*b*) or of an intermediate tinge (*c*), the last being due to the fact that only some of the cells in the parenchyma contained chlorophyll.

In the spring of 1911, the Director of the Carlsberg Laboratory, Dr. phil. Johs. Schmidt, had some purchased fruit, both of the normal and of the mottled variety, sown for experimental purposes. The investigation of the genotypical value of these forms, which was one of the objects in view, was then entrusted to me, on my appointment as his Assistant in August of the same year. The results of the investigations, which extended over 6 years — but comprising only 5 generations, as the plants were not cultivated in 1912, — are extremely clear, and agree entirely with those of Correns' experiments (1908, 1909) with a white-mottled variety of *Mirabilis Jalapa* and Gregory's (1915) with *Primula sinensis*; i. e. they show, that the quality of "mottled-white" in the species concerned is only transmissible through the mother plant.

Only one of the experimental series need here be quoted, and the genealogy of this will be seen from the following scheme, in which I have for the same of convenience omitted the registration marks of the plants, as also the number of individuals, which last amounted as a rule to 100-300 each year.



In this genealogical table, the terms "mottled" and "green" indicate that all fruits of the sowing in question produced mottled or green plants as the case may be, the offspring being throughout homogeneous as far as regards this quality. The signs ♂ and ♀ denote that specimens of the sex indicated were utilized in the manner noted for further cultivation. The experiment particularly shows, that a normally green female plant of *Humulus Japonicus* pollinated by a mottled male produced green offspring, and that the female offspring, even though crossed for several generations in succession with mottled males, remain normal throughout. It was further found, that mottled female plants crossed with green males gave mottled offspring. As regards this last point, however, fuller proof could be obtained by other series of investigations.

A priori, it might be presumed that apogamous fruit formation would be responsible for the fact that the male plants exerted no influence whatever upon the offspring in respect of leaf colour; that this is not the case, however, I have sufficiently proved by a cytological investigation, and by isolating unpollinated clusters of female flowers (Winge 1914). Another explan-

ation must therefore be sought, and it is evidently this (Correns 1909; Baur 1911) that a male plant does not contribute the same amount towards fertilization as the female. The egg in higher plants, as also among animals, contains a quantity of cytoplasm, whereas the male gamete is an extremely small organism, the nucleus of which has but very little cytoplasm or none at all. If no cytoplasm and no plastides from the pollen tubes are carried over into the germ, then it would be natural that the character of the egg cell, and thus also of the mother plant, should in certain respects exclusively determine that of the offspring. We have, however, still not arrived at any thorough understanding of the heredity of mottled plants, inasmuch as it is not yet clear what are the rules according to which the mottled pattern is distributed throughout the vegetative portions of the plant. Even the most highly mottled plants exhibit portions of leaves apparently normal in appearance, and it must therefore be concluded that a vegetative segregation takes place in the ontogenesis of the plant itself — either by suppression of the chloroplasts in certain cells, or by unequal distribution of the same among the daughter cells during the growth of the plant.

The question as to hereditary transmission of qualities through the medium of the cytoplasm is of considerable interest, but has not yet been sufficiently elucidated. In all probability, there are considerable differences in this respect, according as the cytoplasma and plastides from both sides enter into the germ or not. Nichols has (1910) in *Juniperus*, and Coker (1903) in *Taxodium*, demonstrated the presence of starch in the male sexual cell. Strasburger also (1884) found in the case of *Juniperus*, and Saxton (1913) in that of *Tetraclinis*, that the sexual nuclei are surrounded by a sheath of starch at the stage when their fusion takes place; these writers suppose that the starch is derived from the cytoplasm of the male cell, since no starch whatever was visible in the egg prior to fertilization. True, they were not able to prove the existence of starch in the mature male sexual cell, but there can be no doubt that they have, by direct observation, sufficiently proved that starch grains, or specially amyloplasts, can be transmitted from the male plant on fertilization.

The *Oenothera* hybrids, which have been dealt with by so many investigators, and particularly De Vries' double-reciprocal hybrids between *O. biennis* and *O. muricata* (1911) are of peculiar

interest, inasmuch as the heredity studies have been accompanied or followed by cytological investigation of the material (Goldschmidt 1912, Renner (1914). The two reciprocal hybrids are in F_1 both patrocline, and on crossing the reciprocal hybrids one with another the offspring becomes entirely one-sided, all influence from the one of the parent plants having apparently disappeared.

(O. biennis × *O. muricata)* × *(O. muricata* × *O. biennis)* thus gave only *biennis* forms, while on the other hand

(O. muricata × *O. biennis)* × *(O. biennis* × *O. muricata)* resulted exclusively in *O. muricata* forms.

Goldschmidt suggests in explanation of this, that when mutual fertilization of *Oenothera* species takes place, their nuclei cannot exist in the same cell (the embryo cell). The female nucleus would then be eliminated, and in consequence, only the cytoplasm of the female plant exerts any influence on the offspring, which therefore more resembles the father. Goldschmidt claimed to show, from countings of the chromosomes, that one of the haploid nuclei had perished — and a study of the shape of the chromosomes, which differs in the two species, proved that it was those of the father which persisted. Later, the chromosomes doubled again, whereby the chromosome number once more became normal. Goldschmidt therefore opines that the heredity is essentially dependent upon the nucleus, but that the cytoplasm also counts for something in itself. Godlewski (1914) likewise holds this view. Goldschmidt's hypothesis and the results of his investigations have, however, already been challenged by Renner (1914), who denies that the chromosomes of the mother plant perish, as he finds 14 chromosomes in the sporophyte, or at least considerably more than 7, which is the haploid figure for *Oenothera*. On the other hand, he furnishes more interesting data with regard to the offspring material, which, even where segregation takes place, may yet become homogeneous by the suppression of several zygotes. We cannot, however, pursue this side of the matter further, as it does not directly concern our present subject.

The statements concerning hereditary influence of the nucleus and cytoplasm respectively upon the offspring are, as indicated, conflicting. Derschau (1914) has recently expressed himself in favour of the view that the nucleus is in this respect a far more

important organ than the cytoplasm, which latter he considers chiefly as "Transportwege" for matter to the nucleus. That this last cannot be the case will be seen from my previous exposition, according to which the cytoplasm must be regarded as the fundamental organ of the cell, the nucleus being phylogenetically of later date.

I find then, that there is much to support the theory that the nucleus itself, and especially the chromosomes, form the essential morphological basis for hereditary transmission; on the other hand, it cannot be doubted that the cytoplasm is likewise of importance.

In the field of zoology, much has been contributed by experimental investigations; here also, however, the various writers have arrived at different results.

Boveri (1895) tried shaking up the eggs of *Sphærechinus granularis* in a test tube, until several of the eggs were broken into small pieces, some containing nucleus and others not, part of the eggs at the same time remaining whole. He then added to the mixture sperma of *Echinus microtuberculatus*, and the eggs then developed to the pluteus stage. The result showed, that three different forms were discernible; partly common hybrids, derived from the fertilization of whole eggs, partly hybrids of reduced size, from fertilization of egg-fragments containing nucleus, and others again, likewise of reduced size, possessing apparently only the qualities of the father, i. e. of the *Echinus* type. From this, Boveri considered it justificabie to conclude that the cytoplasm does not exert any hereditary influence, as the last-named larvæ, resembling only the father, must have been derived from egg-fragments containing no nucleus, which had been "fertilized" by the *Echinus* sperma.

Godlewski jun. (1904, 1906) has made similar experiments, but takes a different view. He divided up *Echinus* eggs in a similar manner, and isolated the fragments having no nucleus, fertilizing them subsequently with sperma of the crinoid *Antedon*. The offspring here, it is true, did not develope any skeletal structure, attaining only to the gastrula stage, but it could nevertheless be shown that also the qualities of the mother were present to a certain extent. In this case, so Godlewski concludes, the qualities in question must have been transmitted through the cytoplasm of the egg.

The experiments mentioned have one disadvantage; that the hybrid offspring were extremely young, and it is thus perhaps not impossible that some after effects may have taken place in the egg fragments.

Nevertheless, bearing in mind that fertilization in the higher plants entirely corresponds to the copulation of many lower organisms, e. g. green algæ, where undoubtedly plastides from both gametes can enter into the zygote, we must consider it certain that the case of the higher plants can but present a difference of degree, and that in these also, we cannot deny the hereditary influence of the cytoplasm on the offspring. My own experiments likewise bear this out.

I now proceed to consider the behaviour of the chromosomes themselves during fertilization, and will here endeavour — on the basis of what we know as to the nature of chromosomes generally — to put forward some theoretical observations on their importance as bearers of hereditary dispositions. I would point out, however, in the first place, that the cytoplasm must, as already indicated, also be credited with some powers in this direction, and in the secondly, that "predispositions" in the chromosomes cannot of course be supposed to be present as morphologically demonstrable units, but must rest on such chemical conditions in the chromosomes as lie far beyond the range of our present knowledge. That the separate chromosomes may be individually different in structure is another matter.

Starting now with two philozygotic gametes, e. g. two gametes belonging to a plant such as *Sisymbrium strictissimum*, the haploid chromosome number of which is 8, then on fertilization (alien fertilization) a germ with 8 + 8 chromosomes in the nucleus will be produced. Each chromosome from the male sexual cell has its partner among those of the egg cell, but the 16 chromosomes remain individualized, although the mutual attraction between the partner chromosomes may occasionally find visible expression during the diplophase in the form of paired prochromosomes in the somatic cells. Not until the gonokonts are formed — considering now the pollen mother cells — do we find in the prosynapsis an intimate parallel conjugation between the chromosomes from the ♀ and those from the ♂ respectively. The importance of this pairing must, I imagine, be that the

chromosomes two by two supply each other with missing chromatic parts, possibly ides or pangenosomes (Strasburger 1906) in case such should have been lost during the ontogenesis of the sporophyte — and which are necessary if the organism (species) is fully to retain its disposition, i. e. remain genotypically unaltered. That such "loss" must frequently take place, I have already mentioned (p. 143). During the synapsis and subsequent stages, the chromosomes are again separated, and become once more fully individualized during diakinesis. The chromosomes have then undergone this alteration: they have to a greater or lesser degree effected an interchange of substances, so that while each in the main still has its original composition, all are none the less affected by the temporary pairing in the points where they have individually suffered loss during the ontogenesis of the double organism.

The chromosomes which are separated after reduction division — we need here consider only a single pair, A and a — will thereafter no longer be A and a, but A_{1a_1} and a_{1A_1}, A_1 and a_1 indicating, that during ontogenesis a change has taken place in the qualities of the chromosomes, and thus also of the gametes, chiefly through loss, while the sub-indices a_1 and A_1 indicate the mutual influence exerted during pairing of the chromosomes. — We must now presume that the changes which have taken place in the gametes are as a rule extremely slight, the chromosomes passing almost unaltered through the many nuclear divisions, the altered formula for the gametes only denoting a change of quality so extremely small that it can as a rule not be demonstrated at all. Theoretically, however, the newly formed gametes are at least at times "impure". Let us consider this point further.

Taking again two gametophytes belonging to the same species of plant, and considering especially the sexual nuclei, we find that these are, as regards the number of chromosomes, entirely alike, although of different origin. Each chromosome in the male nucleus has a corresponding one in the female. The genotypical differences normally present are due solely to differences in the component parts of the chromosomes in the two nuclei. — It will here suffice to consider a single chromosome in the female nucleus and its homologous fellow in the male, altogether disregarding the segregations which will arise when whole chromosomes are interchanged during reduction division.

The female chromosome will be characterized, for instance, by the following components:

a b c d e f g h i

and the male by a x c d e f g y i

Only two units are different, b and h in the female nucleus being represented by other units in the male, to wit, x and y, whereby the cells become genotypically dissimilar.

On fertilization, all that now happens is that the two chromosomes both enter into the germ nucleus; but when the sporophyte has reached the reduction stage, where new gametes are to be formed, something will have been lost during the numerous mitoses, and the homologous chromosomes under consideration will now have a slightly altered apperance. We can here imagine several cases, according as one or another unit has fallen away.

The male, or the female chromosome respectively, might for instance in the nucleus of a spore mother cell present the following appearance:

Female chromosome: a b - d e f g h i
Male chromosome: a x c d e f - y i

Each of the chromosomes has thus lost one unit, c and g respectively, but in the subsequent prosynapsis, the pairing of the chromosomes takes place by parallel conjugation, and I presume then that the two chromosomes will mutually replace what each has lost, the male chromosome giving off parts of its c to the female, and the latter some of a unit g to the male. Thereafter, the (parent) chromosomes again separate, and will — as it is seen — have exactly the same appearance as when they entered upon the common zygote formation.

Theoretically, then, the gametes might thus easily become "pure" and the segregation complete.

Suppose, however, the appearance of the chromosomes in the spore mother cell to be thus:

Female chromosome: a - c d - f g h i
Male chromosome: a x c d e f - y i

then the units e and g can, by mutual replacement, easily be formed again; with b, on the other hand, this is not the case, as the male chromosome does not contain this unit at all. We

must therefore presume that the male chromosome in prosynapsis will deliver to the female material of the corresponding unit x, so that the chromosomes in the diakinesis when anew separated, will have the following composition:

Female chromosome: a x c d e f g h i
Male chromosome: a x c d e f g y i

The gametes — here only the female — have thus during the sporophytic phase undergone alteration: dispositions have been transferred from the one to the other. The segregation will consequently be impure, though how far it may be possible to discover the fact is another matter. As more or fewer units are lost, a greater or lesser change will have taken place in the gametes, but probably only greater changes, or such as particularly affect the external morphology, will be discernible in practice.

Finally, we have to consider a third possibility, which may be expressed thus:

Female chromosome: a b c - e f g h i
Male chromosome: a x c - e f g y i

The two chromosomes have here lost the same qualities, and units, and are thus unable mutually to replace these. It will then probably depend upon the nature of the dispositions represented by such units, whether the gametes are capable of continued existence or not.

My supposition that the chief importance of fertilization — or rather, of the alternation of generations — is the mutual supplementation of the gametes is, I consider, a very probable one. I can find nothing to prevent the acceptance of such a theory, and believe that the Mendelian laws of segregation and deviations from the same, as well as Johannsen's "pure lines" and other fundamental rules of heredity may well permit, or even support such an hypothesis.

I imagine, that as a rule only few and slight "losses" will take place in the course of each gametophytic or sporophytic ontogenesis respectively, so that they will in most cases be fully compensated for during the chromosome conjugation in prosynapsis. Generally therefore, "pure" i. e. unaltered, gametes may be produced after reduction division, viz: the two parent gamete types, and even if some interchange has taken place,

there will nevertheless arise chromosome-types corresponding in the main with those of the parent gametes, but — after the intermingling during the diplophase — distributed by chance on the gametes. In practical experiment, it is difficult to get at any direct visible expression of this, since the gametophytes of the higher plants cannot as a rule be subjected to so close control, owing to their smallness, as the sporophytes. Altogether, our heredity researches would doubtless gain by the increased employment of organisms with more highly developed gametophytes, such as for instance *Hepaticæ*, in genetic investigations. But my view can evidently very well be brought to agree with the Mendelian laws on the one hand, and with the frequent apparent deviations, great and small, from the same on the other.

As regards the pure lines, we must here of course consider the male and the female chromosomes as being altogether alike in structure. On considering a single pair of chromosomes, for instance, it may be characterized, let us say, by the following qualities:

Female chromosome: a b c d e f g h i
Male chromosome: a b c d e f g h i

When, in the course of ontogenesis, certain units are lost by the male chromosome, it will in the subsequent synapsis have these replaced by the female, and vice versa. As the gametes are throughout entirely homologous, no new units will ever be transmitted from one to the other during chromosome pairing, but if both chromosomes should lose the same unit, then the zygote produced will be genotypically altered, and will then probably represent what we know as a retograde mutant.

Naturally, according to this theory as to occasional loss of genes during developement of the plant, such losses will also be able to occur in plants subjected to continued vegetative cultivation. And as a matter of fact, we have from practical experience several instances recorded of such degeneration of the clones, e. g. the Gravenstein apple, and though it may be difficult to form a well founded opinion as to the value of practical investigators' statements regarding such points, where quite other features — especially pathological — may frequently be the cause of the "degeneration" in question, it seems to me nevertheless quite believable that selection may take place, if the

"sort" shall be maintained throughout a long period; and in reality, such does also involuntarily occur.

The well-known and much discussed experiments carried out by Hansen (1906) with different species of yeast, which lost their power of spore formation, can, I think, very well be included under the same category. The very loss of this power of sexual propagation might be thought to be irreparable; we know, however, still too little as to the alternation of generations by the organisms in question to permit thorough discussion of the point.

That inbreeding can also lead to unfortunate results, as is proved by instances from both animal and vegetable kingdoms, corresponds extremely well with my theory, as the closer relationship between gametes will naturally often preclude the mutual replacement of loss in the chromosomes, which, owing to their common derivation, lack the same units. The ill effects of inbreeding should thus be due to retrogressive mutations. The biotype which arises by mutation in Johannsen's (1915) homozygotic *Hordeum* line, and which exhibits pronounced breaks in the ear, might, for instance, be included here — unless the mutation could have arisen through adulteration of the *Hordeum* material by alien pollination, which is not, I imagine, altogether impossible. In any case, it is perhaps going to far to ascribe the mutation, as does Johannsen, to a sexual cell in particular.

Many plants, however, continue to develope and flourish even when selfpollination constantly takes place through many generations, just as various sexually abnormal individuals, of *Taraxacum,* for instance, thrive excellently by purely vegetative propagation. If such instances be adduced as an argument against my observations on the significance of alternation of generations, I would again point out, as before, that a clone or a definite biotype may possibly retain its qualities for a long time — especially when selection takes place, and it is perhaps only reasonable, that nature should exert a selection among the manifold "vegetative" individuals, for instance in *Taraxacum.* Undoubtedly the less adapted, or the abnormal, here also perish in the struggle for life.

Not least does the theory appear to agree with the fact that abnormally sexed plants are often the very ones which in nature are represented by innumerable forms, e. g. *Taraxacum, Hieracium, Rosa, Alchimilla.* This is, I must suppose, connected with the

fact that occasional hybridisations — as mentioned elsewhere — involve the formation of apogamous or at any rate sexually abnormal "new species", and that these new biotypes and their offspring themselves persist as special minor species or biotypes, for the very reason that their organisation in itself excludes them from fertilization. and thus also from the compensating effect which alternation of generations, in my opinion, produces.

The accuracy with which the separate plant species are able to maintain their genotypical constitution, by retaining, for instance, the chromatic units throughout manifold nuclar divisions, is naturally not always of the same degree, and though it cannot be taken for granted, yet it is doubtless possible that self-pollinating plants are the very ones which in this respect stand nearest perfection. Nature doubtless varies here as in all other features.

I have still a few words to add regarding the individuality of the chromosomes.

Bearing in mind the high degree of constancy exhibited by the number of chromosomes in propagating nuclei, and the fact that the persistency of chromosomes in the somatic tissue is proved; furthermore, that individual differences of size and shape have been observed in the chromosomes of various species, it will be clear that the chromosomes must simply be considered as individuals, inasmuch as each chromosome in the cell is the bearer of certain genotypical dispositions. How the genes are distributed in the chromosomes it is impossible to say as yet, as we have not yet arrived at the empirical foundation for discussion of the point. It must here be borne in mind that the genes are not literally speaking bodies in the chromosomes. The genes are a theoretical term, which may, it is true, have its material equivalent in the nucleus, but the chromosomes consist exclusively of chemical substances.

Johannsen (1913) is opposed to the hypothesis that segregation phenomena and new combinations should be explained by the behaviour of the chromosomes during reduction and fertilization, and he expresses this as follows (p. 600): "..... man hat .... früher daran gedacht, die Faktorenspaltungen und Neu-

kombinationen nach Kreuzung durch Chromosomentrennungen und Neukombination zu erklären. Indem aber wohl immer viel zahlreichere genotypische Faktoren als Chromosomen in einer Gamete vorhanden sind, ist der angeführte Gedanke aufzugeben. Und die Koppelungserscheinungen helfen auch hier nicht; schon weil Koppelungen zuweilen gar nicht absolut sind. Jedenfalls müsste demnach ein "Austausch" von Elementen zwischen je zwei gepaarten Chromosomen z. B. in der Synapsis angenommen werden — dabei geht aber die Individualität der Chromosomen verloren!«

As will be seen, Johannsen here touches upon the possibility of interchange between the chromosomes in pairs, but does not find the hypothesis in question acceptable. As regards the arguments advanced, however, I must point out: in the first place, that no one has yet demonstrated experimentally the presence of more pairs of simultaneously and independently mendling qualities than are expressed by the haploid chromosome number of the plant concerned — as noted by Spillman (1908) and Baur (1911); and in addition, I cannot admit that the individuality of the chromosomes should necessarily be lost, even though interchange between them may take place in prosynapsis and synapsis. The term "Individuality" is, it is true, difficult to define in this connection, but the cytologists who have demonstrated the individuality of chromosomes, really mean only the "persistency and retaining of formal peculiarities throughout the generations". The qualitative constancy — or individuality, — cannot of course be studied under the microscope. The position might be illustrated, for instance, by pointing out that two ships at sea without losing their "individuality" might well exchange certain wares in order to complete their stores; the chromosomes can doubtless carry on a similar traffic during their conjugation in the synapsis stage.

Janssen's theory (1909) of Chiasmatypy is just intended to explain how the qualitative individuality of the chromosomes may be thought to undergo alteration through the mutual interchange of chromosome parts, without loss of their characteristic form. — The theory in question is very neatly constructed, but doubtless superfluous to the cytologists, since the chromosomes are of such a physical character that whorls and curves may well be thought to arise in other ways than those suggested by the

chiasmatypy hypothesis. To experimental heredity research, the theory proved more welcome. But we involuntarily ask, by what rules does chiasmatypy take place, or not take place? Is it of purely spontaneous occurrence, then we still lack the basis for comprehension of the occasional deviations from the coupling theory which it should explain. — It is characteristic that the heredity investigators have endeavoured to give the theory of chiasmatypy a wider range than the cytologists themselves, by supposing a double and treble "crossing over" and "interference". Morgan (1911, a, b), and his disciples, especially Sturtevant (1915) have furnished most important contributions to the knowledge of coupling qualities. Especially attractive is Sturtevant's demonstration that the genes may be imagined as arranged on a line — a theory which perhaps has its earliest origin in Roux' work (1883), works by Correns (1902) and De Vries (1903), and particularly by Sutton (1902), who was the first to advance the theory that the genes mendled independently on account of their different position in the chromosomes, and that their respective place in the line is decisive for the percentual frequency of the coupling. Nevertheless, the theory should hardly be applied literally to chromosomes. In the first place, we have no cytological proof that this is permissible, and further, the whole construction of the theory is very closely related to the simple mathematical principle which permits us, given the relation between A and B, and that between A and C, to calculate thence the relation between B and C, so that other theories will probably also suffice to meet the case. We cannot, however, go into this question here.

The doubling of the chromosome number, which has been generally accepted by the cytological investigators, who found that certain species in a sphere of relationship had twice as many chromosomes as others — should, as I have pointed out in detail, in my opinion be explained in an essentially different manner from that hitherto followed; not as a cleavage of the chromosomes, but as indication of hybridization, and thus an addition of the chromosomes.

That regular reduplication nevertheless can also take place is beyond doubt, and has been shown to be the case by experiments already quoted (Él. and Ém. Marchal and others). But

that such reduplication should be of any considerable importance for phylogenetic problems I cannot believe. The size of the cells is adapted, as we know, in a certain degree according to that of the nucleus, especially according to the number of chromosomes, and it is thus only reasonable, as experience also has shown, that there should, in respect of size of the individuals, often be a distinct plus for the polyploid individuals as compared with the normal diploids; but the chromosomatic units are and remain of the same nature, only their number of mass being doubled; the genotypic values thus continue unaltered[1]). If then, the chromosome number of a plant be from any cause really doubled by a splitting of chromosomes, then we have a quantitative, but not a qualitative difference, just as 1 molecule NaCl + 1 molecule NaCl continues to be NaCl.

Finally, I would further point out that fundamental principle for the views here advanced is closely allied to our knowledge of the fact that cellular divisions do not always proceed with infinite correctness and certainty, so that the genotypical value of the cells must be supposed to alter more or less in course of time. The alternation of generations here comes to the rescue, permitting as it does a continual supplementing of elements of hereditary importance which have been lost, whereby the genotypic value of the species gains in stability. The theoretic difference of the pure lines from heterozygotic material is not affected by this. As a principal argument for the view that irregularities occur in cellular division, I must quote the fairly frequent occurrence of abortions in nature, as also the fact that experimental investigations have fully demonstrated the influence exerted by external conditions upon the course of cell- and nuclear division.

[1]) It may perhaps be maintained that the genotypic values really are altered when individuals have become hereditarily stamped in regard to size. We here touch upon an interesting but difficult problem As we know, the size of the individual may well be genotypically determined. It is, however, natural to suppose that quantitative qualities may perhaps have their hereditary foundation in quantitative differences in the chromosomes.

Winkler's work on experimentally produced tetraploid *Solanum* forms (1916) and similar ones with *Oenothera* show, that a certain change of form also does take place. We may doubtless suppose that the plant, owing to the larger cells, will for physiological and mechanical reasons be unable to shape itself altogether in the fashion of the diploid plant. When the volume of the cells is doubled, this does not involve a doubling of the surface extent, and it is therefore but natural that the individuals should become coarser and of a stronger green colour.

CHAPTER 7.

CHROMOSOMES IN HYBRID ORGANISMS.

The teratological phenomena have always been of great importance to organography — and as the cytologists often encounter macro- and microscopically discernible phenomena of related nature in hybrid organisms, it is only natural that the study of heterogeneous fertilization and all that it involves should be particularly valuable in the study of chromosomes.

Among plants, specific hybrids are as a rule sterile; indeed, imperfect pollen formation in flowering plants may often be a criterion of their hybrid origin (Juel 1901 and others). In *Syringa Rothomagensis*, a hybrid between *S. vulgaris* and *S. Persica*, Juel found poor pollen developement. Remarkable in this case was the fact that the parent plants also had but a very inferior power of pollen formation, the former producing only about 50 % good pollen grains, and the latter still fewer — though it is not known whether the parent plants may likewise have originated as hybrids. I have also previously mentioned several plants, for instance *Callitriche* and *Hemerocallis*, in which pollen irregularities take place, although it has not been proved that these species were of hybrid character.

On the whole, however, imperfect reduction is associated with the hybrid nature, but it would be only reasonable to suppose that also other causes might affect that stage in the alternation of generation which must doubtless be considered as the most delicate.

That the "striving" of the hybrid plant to complete the cycle of the alternation of generations may also be stopped at an earlier stage has been shown by Tischler (1906, 1907, 1908) and Janczewski (1909) who found, in *Ribes* hybrids, *Syringa* and *Mirabilis*, that the tapetum of the anthers became abnormally developed, so that it was evidently unable to nourish the pollen mother cells. I have, however, found a similar state of things in a monoecious individual of *Humulus lupulus* (Winge 1914) from which it would seem that internal discrepancies of another nature can also produce the same phenomenon.

The life cycle of sterile hybrid individuals — both in plants and animals — appears to be regularly interrupted either at so late a stage as in the above-mentioned instances, i. e. on account

of the imperfection of the sexual organs — or in plants, especially of the spore formation — or at an extremely early stage, soon after the sporophyte has been formed by the hybrid fertilization, and that there are thus two critical stages. Once the hybrid has passed the first test, which decides whether it is capable of independent vital action at all, it developes with often surprising luxuriance, until the inadequacy of the sexual products put an end to its further propagation, and the biotype produced dies with the individual.

The great majority of spontaneously produced hybrids probably die at so early a stage in their developement that they are never recognized as such; numerous "parthenocarpous" phenomena and imperfect embryos in the vegetable kingdom should, in my opinion, be regarded as derived from heterogeneous crossings.

Simultaneously with Tournois (1914) I pointed out (Winge 1914) that *Humulus lupulus*, when pollinated with *H. Japonicus*, forms apparently normally developed fruits, containing, however, only an extremely small embryo, which never attains a sufficient degree of developement to permit of its producing an independent plant. Tournois had previously (1911) in like manner crossed *Humulus lupulus* with *Cannabis sativa*, and found, in this case also, a small embryo formation. The question is, then, whether we have here a really normal fertilization, or whether the heterogeneous pollination only occasions a more superficial stimulation, which might cause the female sexual cell in *Humulus* to develope parthenogenetically into a small embryo. Tournois is of opinion that this is most often the case as regards the *Humulus* × *Cannabis* crossing, but he considers *Humulus lupulus* × *H. Japonicus* to have arisen by true fertilization. The first-named case I have not further investigated; as regards the latter, however, I was able to show (l. c.) that the pollen of *H. Japonicus* germinates in normal wise on the styles of *H. lupulus*, after which the pollen tube grows in precisely the same course down to the embryo sac as the pollen tubes of *H. lupulus* itself. Fertilization seems to be continually proceeding — and so far, Tournois' and my results agree. *Cannabis* has not been sufficiently investigated to permit of any decisive conclusion; *a priori*, however, there seems to me but little grounds for regarding the two cases as different in principle.

The fruits produced by crossing *H. lupulus* × *H. Japonicus* are, as mentioned, of normal size, and the inflorescenses (the hops) develope strongly, as in a normally pollinated plant. In accordance with the small size of the embryo, the weight of the fruits is, however, far inferior to that of normal hops, the ratio between the weights (specific gravity) in my experiments being as 3 : 10, and undoubtedly we should, if unacquainted with the true state of the case, declare the fruits to be parthenocarpous, the embryo being hardly visible, even under the lens.

As we know, *Humulus lupulus* has often been regarded as occasionally parthenogenetic or apogamous (Wettstein 1903 and others). That this is not the case is sufficiently shown by Tournois' and my own investigations, inasmuch as the experiments throughout gave negative results.

It is commonly asserted among practical hop growers in Germany and Austria, where the male hop plants are kept down, or exterminated as far as possible, for the sake of cultivation, that hop fruits are nevertheless often found in the hops. To find an explanation of this, I was forced to incline to the belief that not only could wind pollination at long distances occasion this, but also that *Humulus lupulus* might be fertilized by more distantly related plants.

In 1883, a Bavarian hop-grower, writing in "Allg. Brauer u. Hopfen Zeitung", Vol. 2, p. 630, put forward the view that stinging nettles apparently were able to fertilize the hop, so that the latter formed fruits. In 1914, I stated, on the basis of my own experience up to that date, that this might doubtless be correct, and in the summer of 1914 and 1915, I carried out a series of experiments with heterogeneous pollination of *Humulus lupulus* in the experiments nurseries of Carlsberg Laboratory. The results are of no slight interest, although up to the present they are not supported by any cytological investigations.

A couple of hundred transparent bags were placed round the hop bines with the flower clusters not yet in bloom, and when these were at the stage where pollination could take place, they were treated in various ways, some being pollinated by means of a brush with pollen collected from various plants not too distantly related, while others were chemically stimulated with the view of inducing, if possible, parthenocarpous developement, or perhaps parthenogenetical embryo formation.

I tried the following:

	Embryo developement
1. Pollination with pollen of Urtica dioica	Fairly good
2. » » » » Urtica urens	Extremely strong
3. » » » » Cannabis sativa ..	Good
4. » » » » Pilea cynocrambe[1])	Some few small embryos?
5. » » » » Peperomia resedæflora[1])	Some few small embryos?
6. Etherisation (varying doses).....	Negative result
7. Treatment with hydrous extract of hop-pollen	Negative result
8. Continued isolation (control)	None.

The experiments might have been even more comprehensive, but my principal object was to investigate the case of *Urtica*. The embryos produced by pollination with *Urtica dioica* did not entirely fill the ovarial cavity but the result of the pollination with *Urtica urens* was alogether astonishing. The hops contained extremely strongly developed fruits, surpassing as a rule in size and weight those usually found in well-developed normal hops. The embryo was apparently normally developed, and closely rolled up, white and firm. It was with the greatest interest that I sowed these fruits in the spring of 1915 — about the first of April — together with numerous hop fruits derived from homogamous pollination of *Humulus*. as generally sowed in the experimental nursery at Carlsberg Laboratory. The hardy appearance of the embryo seemed to promise that it would thrive well, and I naturally expected that the seedlings would resemble those of *Humulus*. since the embryos in question were rolled up, whereas the embryo in *Urtica*, as we know, is straight.

The result showed, that not a single fruit was capable of germination, while the normal hops, subjected to precisely the same conditions, grew most satisfactorily. Observations made from time to time showed that the embryo died; after a while, they commenced to become soft, and turned a greyish yellow colour, indicative of incipient decay. The fruits derived from pollen of

[1]) I was only able to procure a very small quantity of this pollen from the Botanical Garden, as the flowers in question are extremely small, and only a few of the flowers in a cluster are in bloom at the same time. Only two flower-bearing branches enclosed in bags could therefore be pollinated.

Urtica urens and *Cannabis sativa*, however, did not lose their fresh appearance until after three months had elapsed.

The explanation of this mystery must, I take it, be that a true heterogamous fertilization had taken place, and that the hybrid had been capable of developement as long as it was nourished by the mother plant, but was from lack of inner consistency incapable of independent action. This view is supported by well known analogous instances in the animal kingdom, to which I shall later refer, where heterogamous fertilization between organisms likewise belonging to different genera and families in the system have resulted in a speedily terminated hybrid embryo developement. Cytological-anatomical investigations of the supposed hybrid embryos will naturally afford a solution of the question, and such should therefore be carried out. I hope to have an opportunity of pursuing the matter.

I should point out that one of the reasons which prevents me from accepting the view that the heterogamous pollination has only been a stimulation to parthenogenetic — possibly apogamous — embryo developement in the hop is that it might in such case be expected that the various branches pollinated with heterogamous pollen, on the same plant, would all have developed equally large embryos, but this was not the case. The pollination can therefore hardly have been a stimulation, giving rise to a process in itself having no connection with the alien pollen employed.

Analogous cases are, as indicated above, afforded by the zoological literature; it will here suffice to mention some few heterogeneous crossings between genera more or less distantly related.

At the zoological station in Wimereux, Giard (1900) succeeded in getting eggs of *Psammechinus miliaris* to develope to a certain degree on fertilization with sperma of *Asterias rubens*, while Loeb has obtained a like result with ophiuroid sperma. Godlewski (1910-1914) has found, that the combination *Echinus* × *Strongylocentrotus* gives a so powerful embryo developement that nearly 100 per cent plutei are formed. The reciprocal crossing, on the other hand, resulted in a far smaller and greatly decreased power of developement. He has also shown that the sperma of the annelid *Chætopterus* and the mollusc *Dentalium* incite developement in echinid eggs. He attempts to explain

such heterogamous embryo developements, or rather their imperfection, in the following (linguistically awkward) manner (1910-1914, p. 879): "Meine Erwägungen führen also zu der Annahme, dass in der Regel die heterogene Befruchtung deshalb keine normale, länger dauernde Entwicklung ergibt, weil das fremdstammige Spermatozoon keine entsprechenden Mittel, resp. nicht eine hinreichende Mengen in das Ei einführt, dies es ihm erlauben würden, die Oxydationsvorgänge richtig und dauernd zu regulieren, die Kernsubstanzsynthese, besonders die Nukleinsäuresynthese durchzuführen, und infolgedessen vergiftet sich das Ei resp. der Keim selbst. Er wird also nicht vom Spermatozoon, sondern durch eigene in ihm sich abspielende Prozesse vergiftet."

In some hybrids pertaining to zoology, the presence both of the mother's and of the father's chromosomes can be discerned, as these at times differ so markedly in appearance as to render distinction possible; in other cases it is found that some few or all of the chromosomes from the one parent are ejected from the nucleus during division of the same, and remain lying in the cytoplasm. In the hybrid *Arbacia* × *Sphærechinus*. Baltzer (1910) found for instance an elimination of 18 chromosomes, presumably all those of the father, and the hybrid embryo is then, accordingly smaller than that of the pure species. Under such circumstances it may be justifiable to speak of an intermediate stage between hybrid and parthenogenetic developement.

The most interesting side — from a cytological and genetic point of view — of heterogamous fertilization is the study of what takes place with the chromosomes in the hybrid offspring.

The classical investigations of Rosenberg with the hybrid *Drosera longifolia* × *D. rotundifolia* (1909 especially) showed that the hybrid plant contained 30 chromosomes in the somatic cells, this being the sum of the haploid number in both parents, which had respectively $x = 20$ and 10. In the hybrid gonokonts, the chromosomes conjugated in such a manner that the 10 *D. rotundifolia* chromosomes united with 10 of those from *D. longifolia*, the remaining 10 of the latter distributing themselves irregularly throughout the daughter cells in the tetrad division, partly with formation of small nuclei, as already mentioned. On reduction division therefore, it was possible to discern the presence both of paired and unpaired chromosomes, and the gones received 10 of the paired chromosomes, and a varying number of un-

paired. On the homoeotypical division it was noticed that the single chromosomes were as a rule not yet eliminated, but often divided by normal longitudinal cleavage together with the rest, abt. 13 to 16 going to either pole. Unfortunately, the hybrid was only slightly capable of producing offspring, the pollen being sterile, and only a few embryo sacs of comparatively normal appearance were produced. These were fertilized by pollination from *D. longifolia*, and a smaller embryo was formed, in which, as was to be expected, at least 33 chromosomes could be counted, i. e. 20 of *D. longifolia* + 13 hybrid. I could therefore imagine, that *D. longifolia* was produced by hybridization between two species or forms with x = 10, the chromosomes of which had become added together in the zygote, and had then suffered division. On further crossing with *D. rotundifolia*, only half would then be capable of conjugation with those of the latter.

Now Rosenberg (1909) investigating the chromosomes in the apogamous *Rosa canina persaticifolia* and *Rosa glauca Afzeliana* var. *dilatans*. finds, that in the diakinesis (p. 155) "treten sehr deutlich zwei Formen derselben hervor, teils enfache, stäbchenförmige, teils deutlich zu Doppelelementen vereinigte Chromosomen". But we cannot help thinking that the cytological processes here are so markedly similar to those which take place in the *Drosera* hybrid, that they should probably be due to the same cause, viz: hybridization. On further perusal of Rosenberg's description of the *Rosa canina* form, we find, on p. 156: "Nach dem Zählen von etwa 30 Kernen im Diakinesen-Stadium aus verschiedenen Antherenfächern habe ich gefunden, dass die Zahl der Einzelchromosomen ungefähr 20 beträgt, diejenige der Doppelchromosomen gewöhnlich 7, und mehr vereinzelt 6 betrug. Die Zahl der Einzelchromosomen schwankte zwischen 19 und 22. Genau dieselben Zahlen erhielt ich bei der Untersuchung der Diakinese bei der *Rosa glauca*-form." The apogamous *Rosa* forms investigated have thus, according to Rosenberg's statement, about 2x = 32, viz: abt. 20 single + 6 to 7 double chromosomes, whereas the chromosome numbers in normal *Rosa* species are as a rule x = 8 end 2x = 16. I cannot ignore the fact that we have here a state of things closely resembling what is met with in *Drosera* and other hybrids — apogamy being present at the same time. The apogamous plants have here, in the case of *Rosa* — as is usual elsewhere — twice as many

chromosomes as normally sexed species, and I am therefore of opinion that the *Rosa* species investigated by Rosenberg must be due to hybridization between two forms with x = 8, the chromosomes of which have not been able to pair directly, but have by cleavage (see p. 199) produced a set of double chromosomes, in which chromosome pairing originally could take place between all sister chromosomes, but is now only present to a slighter degree. Rosenberg himself inclines to Strasburger's view, that the apogamy must be a result of an extra chromosome division, produced by some cause unknown.

Farmer and Digby (1910) who have investigated the hybrid *Polypodium Schneideri*, found here, on reduction division, 95 to 105 chromosomes (= x) whereas the parent species *P. aureum* and *P. vulgare* var. *elegantissimum*, had x = 34 and abt. 90 respectively. Presumably then, a varying number of chromosomes had here united in pairs. In any case, no doubling of the chromosome number had taken place, as will be seen from the approximate figures; there would in such case have been about 248 chromosomes. Accordingly, as I think we may say, the plant is not apogamous, but sterile. That it should be capable of developement at all is presumably connected with the fact that a certain amount of chromosome binding does after all occur.

Kuwada's investigations with variety hybrids of *Zea May* (1915) call for some mention of this writer's highly remarkable results, which I must confess appear to me altogether incomprehensible. Kuwada finds that the races of starch maize have a constant x = 10, the sugar maize on the other hand normally x = 12. This last figure should, however, be able to vary from 9-14 in one and the same individual, but only 10-11 are at all frequent, and 12 is by far the most general figure. He now finds, that the hybrid has in the somatic tissue 20, 21, or 22 chromosomes — as a rule one of these figures appears constantly in each individual — i. e. 10 starch maize chromosomes + 10, 11 or 12 sugar maize chromosomes. But on reduction division, we are told, there appears the highly peculiar phenomenon, that reduction takes place normally, with normal formation of gemini, so that 10, 11 or 12 gemini can be observed. With regard to the separate chromosomes, it can further be seen that when 10 are present, two of these are always of double size, and where there are 11, one is always of double size, the remainder being

alike in such respect, and finally, when there are 12, all are of more or less same size. How 11 or 12 gemini can be formed at all, when the one sexual cell constantly carries but 10, the other 10 to 12, is more than I can understand, and it calls for some special explanation. Equally remarkable does it seem that the principal quantity of chromosomes should be of smaller size than the 1 or two exceptions. It would be more natural to expect that 10 large (conjugated) and 1 to 2 smaller (unpaired) would be found! We must ask ourselves whether it could here be possible that an occasional ea ly separation of one or two chromosomes could give rise to the apparent presence of more than 10 chromosomes and gemini in the sugar maize, i. e. a situation resembling that already mentioned (p. 183) in the case of *Chelidonium*, or that described by Tschernoyarew (1914) in *Najas major*, where $x = 6$ but $2x$ apparently 14, one pair of chromosomes having broken up into two. I must confess that I find such an explanation unlikely, and will therefore — not without a certain scepticism as to the present results — await further investigation of chromosome conditions in *Zea* hybrids.

Interesting are the hybrids between *Bryonia alba* and *B. dioica* (Tischler 1915) and between *Ægilops ovata* and *Triticum vulgare* (Bally 1912) of which latter Bally found a spontaneously occurring individual in Poppelsdorf with a constant intermediate chromosome number; with regard to these, however, it is doubtless too early at present to express any opinion.

The zoological literature affords important contributions to the study of chromosomes in hybrids, mostly tending in the same direction as the instances known from the vegetable world. The classic varieties *Ascaris megalocephala univalens* and *bivalens*, with $x = 1$ and $x = 2$ respectively, are able to form a hybrid with $2x = 3$; this, however, it has been found impossible to investigate further up to now, owing to the sheer sterility of the intermediate form (Herla 1893). Guyer's studies (1900) with hybrid pigeons gave more positive results. The pigeons of pure species had $2x = 16$ and $x = 8$, these eight chromosomes appearing ring-shaped in the primary spermatocytes, in connection with which there occured both small and large rings. In extreme cases, 16 chromosomes could be discerned, which then lay arranged in two spindles, eight in each, these presumably belonging to each of the parents. As in the case of plant hybrids then,

we find here also an incomplete binding of the genetically different chromosomes.

Highly interesting also are the investigations of Harrison and Doncaster (1914) with geometride hybrids. Two species are here to be considered; *Lycia hirtaria* with $2x = 28$, and $x = 13$, of which latter, however, one is double (at any rate in the male) — and *Nyssia zonaria* with $2x = 112$ and $x = 56$. The chromosomes of the last-named species are far smaller than those of *Lycia*. and, as will be noticed, the chromosome numbers stand in a remarkably simple relation, viz: as 1 : 4.

The reciprocal hybrids differ somewhat. *N. zonaria* × *L. hirtaria* produced exclusively males, with $2x = 70$ in the spermatogonia, of which, as was to be expected, 14 large and 56 small. On the first spermatocyte division, nearly the somatic number is observed, but always 5-15 below this, a corresponding number of pairings having taken place in prosynapsis. The chromosomes do not all divide, and in the second spermatocyte division, abt. 9 large and abt. 42-46 small chromosomes can be counted.

With regard to the reciprocal hybrid *L. hirtaria* × *N. zonaria*, both males and females were found, but only a single individual, a male pupa, was suitable for investigation. The spermatogonia all showed 14 large and 56 small chromosomes, i. e. $2x = 70$. In the first tetrad division, 52-60 were counted, of which up to 18 were large, probably on account of partial pairing; in the second division, there were abt. 10 large and 40 small, about 50 in all. It seems then, as if a slightly large number of chromosomes had here conjugated than in the first-mentioned hybrid.

The hybrids are sterile, and the writers are doubtless correct in stating (p. 245): "The sterility between the two nearly allied species may have arisen simply from a difference of grouping. Since the units are grouped differently, the chromosomes cannot pair properly in synapsis, and this may be the cause of the sterility".

I must still refer to some of Federley's researches on the chromosomes in hybrids — and these are, in my opinion, the most important which have appeared up to now.

Federley's studies on *Dicranura* hybrids (1915 a) which I have recently received, give us a further instance of related chromosomes in related species, *D. vinula* having 21, *D. erminea* 28,

according to which the chromosomatic cardinal number for *Dicranura* is 7. In the hybrid, the number of chromosomes varies on first tetrad division between 29 and 45. This fluctuation is said to depend partly on the fact that a varying number of chromosomes are capable of conjugation, partly on occasional fusion of chromosomes. When such fusion takes place, large lumps of chromatin are produced, and the writer is of opinion that in such case the cause lies in a chromatolytic process produced by antagonistic action of the specifically different idioplastic substances in the hybrid. Plasmolytic phenomena also occur during the second tetrad division, and in the metamorphosis of the spermatids.

Federley states that the irregularities in the hybrid first become apparent during reduction division, as in all probability the relation between the specifically different chromosomes only then becomes intimate, so that the disharmony finds expression.

The investigations with *Smerinthus populi* × *S. populi* var. *austauti* (1915) gave similar results. Here, however, both forms have the same number of chromosomes, viz. 28. In *S. austauti* × *S. populi* the chromosome number in the spermatocytes varied between 28 and 33, some few chromosomes at times neglecting to conjugate.

Federley is now of opinion that the conjugation of a varying number of chromosomes in the different spermatocytes indicates a qualitative difference arising in course of ontogenesis between the chromosomes in question. Federley is here evidently arguing along the same lines as I have endeavoured to make clear in the foregoing; that nuclear division does not proceed with such precision as to maintain the division product constantly equal to its origin. He follows up his victory by asserting that an alteration of chromosome qualities contradicts the theory of individuality in chromosomes[1]) which demands an identical set of chromosomes in all gametocytes of the first order, and he believes that segregations can thus take place independently of Mendel's laws. This last statement I would modify to the expression that deviations from the Mendelian segregation may occur, as segregations in the ordinary sense of the word are produced by a regular distribution of the genes among the

[1]) Federley does not here mean persistency, but true "qualitative" individuality, as he expressly states l. c. p. 20. Ö. W.

offspring; irregular differences in the sets of chromosomes produced by imperfection in the mitotic divisions, can never lead to regular segregation, but only to irregularities, i. e. deviations from what was theoretically to be expected.

Federley considers it possible, that races with for instance 29 chromosomes can be derived from others with 28 by hybridizations, provided the offspring with the imperfect conjugation be fertile. It is doubtless possible that this might take place. That it rarely does occur, however, would seem to be proved by the relation between the numbers of chromosomes which, as I have shown, is continually encountered when we study the question closely, but as an instance corresponding to this may be cited the *Oenothera* forms with 15 chromosomes.

Federley presumed — as did I myself — that the sterility of the offspring is in inverse proportion to the power of conjugation in the chromosomes. This able writer maintains, and not without reason (p. 22) that "die Theorie von der Reinheit der Gameten bildet das Fundament der modernen Genetik. Mit ihr steht und fällt die mendelistiche Vererbungsanalyse".

As to this there can be no question, and that the theory is subject to errors I have also endeavoured to show in the foregoing. The eagerness to apply mathematical laws to the science of genetics has frequently led to neglect of the fact that we have here to deal not with columns of figures, but with living cells, dividing infinitely under influence of surrounding conditions. Investigators have erroneously presupposed the same precision in these as in the elements of pure mathematics, forgetting as a rule that the forces of nature — the environment — which are of no importance to mathematics, have a very considerable effect on the living organism. "Wir müssen die Erblichkeitslehre mit Mathematik, nicht aber als Mathematik treiben" as Johannsen puts it (1913, p. 2) a dictum with which we must entirely agree. Even where the material is pure, there can in genetics only be a question of rules, not of laws in the mathematical sense.

Federley's previous work (1913) with butterfly hybrids, produced some apparently remarkable results, which nevertheless, when well considered, fall in very well with the theory as to the great importance of chromosome pairing for normal sexing. He found that hybrids between *Pygæra curtula* (x = 29) and *P. anachoreta* (x = 30) as also between *P. curtula* (x = 29) and *P. pigra*

(x = 23) were fertile despite the fact that chromosome conjugation was almost or entirely lacking, and no synapsis stage could be discerned. A characteristic diakinesis stage was therefore likewise not found. The fertility was, however, of a peculiar nature, no reduction taking place, so that we are here justified in calling it a somatic sexual cell formation. Apogamy — or parthenogenesis if preferred — did not take place, or was at any rate not observed, as nothing is stated as to the hybrid eggs being capable of independent developement, although 29 + 30 and 29 + 23 chromosomes respectively were present, i. e. the sum of the chromosomes in the parent gametes. The diploid eggs were found to be capable of fertilization, and the organism produced was thus triploid. (*P. curtula* × *P. anachoreta*) × *P. anachoreta* thus produced a hybrid with 89 chromosomes, i. e. 29 *curtula* and 30 + 30 *anachoreta* chromosomes. In this secondary hybrid, a typical synapsis was found. On the first tetrad division, the *anachoreta* chromosomes were reduced, while those of *curtula* on the other hand, underwent an equation division, and the second division was an equation division for all chromosomes, whereafter the spermatides obtained 59 chromosomes, just as in the primary hybrid.

On considering these phenomena from a theoretic point of view, it is beyond doubt that the fertilization between *Pygæra curtula* and *P. anachoreta* must have been not a little imperfect — as mentioned on p. 198. Presumably, a number of chromosomes would have been of so homologous character that some degree of binding took place, as is also shown by the occurrence of some few conjugations in the diakinesis stage, and the sympathy thus expressed gave the hybrid power of existence. But the union of the gametes only gave rise to a product which retained its gametophytic nature, — a double gamete or hybrid gamete with the power of further fertilization. The binding between the chromosomes of *curtula* and those of *anachoreta* would have been so loose that *anachoreta* chromosomes later introduced into the hybrid gamete by renewed fertilization immediately seized on their homologues in order to form a more solid alliance with them, leaving the *curtula* chromosomes to their fate.

Federley inclines to the view that new species may arise by hybridization, and it is only reasonable to suppose that the hybrid *Pygæra curtula* × *P. anachoreta* should in reality be con-

sidered as such, even though it may have but a restricted power of propagation. It would be most interesting in this respect to investigate how the hybrid would behave on homogamous fertilization — whether F_2 would on formation of the sexual cells throw off the gametes of the pure species or form diploid hybrid gametes. Federley states, however, that the pairing of $F_1 \times F_1$ is rendered difficult by the numerous failures.

The chromosome number in itself Federley does not regard as of great importance, and I must admit that the chromosome numbers 23, 29 and 30, which he finds in the mentioned *Pygæra* species, do not suggest any great constancy or indicate relationship between the chromosomes within the sphere of relation. Though it may be possible that elimination of chromosomes can in certain cases break through the rule as to the relationship of chromosomes within systematically natural groups, this cannot affect the facts with regard to the chromosome numbers in the vegetable kingdom which I have demonstrated in the foregoing.

Winkler's *Solanum* "grafting hybrids" need not be dealt with at any length, as each separate chimæra is, according to Winkler's investigations, a double plant, derived from a kind of interweaving or interpenetration of two original species, *Solanum nigrum* and *S. lycopersicum*. It was therefore not surprising that Winkler (1909, 1910) found the chromosome numbers of the species again in the chimæras. *Solanum tubingense* and *S. Gaertnerium*, both of which most resemble *S. nigrum*, have like this species $x = 36$. *Solanum proteus* and *S. Koelreuterianum*, which more resemble *S. lycopersicum* have like this $x = 12$. — Winkler has subsequently (1911) discovered that also the somatic cells likewise continually appeared as in one of the parent species, i. e. certain cell layers in the chimæras having 72, others 24 chromosomes. The matter is thus perfectly clear. The union of the two species in the chimæras has not been of so intimate a nature that any cellular, still less any nuclear union took place. Only in the case of *S. Darwinianum* is there still some uncertainty in this respect.

Cytisus Adami, which, especially since Buder's investigations (1910, 1911) has been found to be of related nature, viz: a periclinal chimæra of such a structure that only the epidermis belongs

to *Cytisus purpureus*, the remaining tissue parts to *C. laburnum*. has, like the pure species, x = 24. Strasburger's supposition (1906) that *C. Adami* should be derived from a crossing between the two pure species cannot thus be correct. We have, moreover, Adam's own assurance that he grafted *C. purpureus* on to *C. laburnum*, and that the peculiar form appeared as an adventive bud at the point of union.

CHAPTER 8.

HYPERCHROMATIC CELLS.

In a previous cytological work (1914) I took up the question of hyperchromatic cells, especially the tapetal cells in the anthers of *Humulus*, which, as in many other flowering plants, generally include two diploid nuclei — i. e. x being = 10, 40 chromosomes — or often, by further divisions, even more.

This example shows, that the cells of the somatic tissue do not necessarily contain 2x chromosomes. As I pointed out in detail at the time, this multinucleate quality in the tapetum has been considered (Rosenberg 1899, Bonnet 1912) as proof that the tapetum consists, phylogenetically speaking, of somatized pollen mother cells, the divisions giving rise to the multinuclear state being regarded as parallel with the tetrad division of the pollen mother cells.

I showed that this could not be the case, adducing as argument inter alia the fact that I had, in investigating the gametophyte of a Cycadea, also here — in the tapetal cells of the archegonium — found hyperchromatic cells of precisely the same nature as in the sporophytic tissue of the flowering plants. Naturally therefore, I was compelled to consider the wealth of chromatic substance as standing in relation to the physiological function of the tapetum, and I cited the fact that hyperchromatic cells with 3x chromosomes are normally found in the endosperm, which itself has a similar function, as conveyor of nourishment, to that of the tapetum.

I can now supplement these observations with some further instances of the occurrence of hyperchromatic cells in the gametophytic tapetum.

Miss Kildahl (1908) writing of the Taxacea *Phyllocladus*

alpinus, writes (p. 344) as follows: "The embryo sac is surrounded with a layer of jacket cells with very large nuclei, and many of the cells are multinucleate". The illustrations accompanying the statement in question, Plate 21, Fig. 21 a and Plate 22, Fig. 31 b show, as a matter of fact, up to 7 nuclei in one cell.

Eames has (1913) observed the same thing in *Agathis australis*, and states (p. 6): "The jacket cells are sharply differentiated from those of the gametophyte surrounding them." . . . . . "A bi- or multinucleate condition is common, particularly in the upper portion, the nuclei being large with often two or more nucleoli".

It is common to find, moreover, in many gymnosperms, that the older prothallium cells are bi- or multinucleate. Saxton (1913) observes, by way of example, with regard to *Actinostrobus pyramidalis* (p. 341): "The older cells of the prothallus are generally binucleate or fournucleate" — and he states that this has been found to be the case in several others, as for instance *Widdringtonia*, *Callitris*, *Podocarpus*, *Taxus*, *Cephalotaxus*, *Araucaria Cookei*, *Cupressus* and more rarely in *Pinus*. The writer in question opines, that the binucleate cells are produced by a mitotic division of the originally mononucleate elements, but that there may possibly, by resorption of cell walls, also arise binucleate cells.

The hyperchromatic cells in vegetable tissue are of particular interest. In the first place, it has been claimed, as we have seen, with regard to the tapetum, that the higher chromosome numbers were due to extra mitoses, arising from atavistic reminiscences in cells which had originally belonged to the sporogeneous tissue. And in addition, the opinion has been advanced (Němec 1910) that reduction division was discernible in such cells, this being otherwise restricted to the gonokont nuclei.

With regard to the first point, I have, I believe, inter alia by the examples quoted, furnished sufficient proof that the hyperchromatic tapetal cells are as a matter of fact not restricted to the sporophyte by any means, but may equally well occur in the haploid generation.

In my paper on the cytology of *Humulus* (1914) I was able to assert that hyperchromatic cells should be considered from a phylogenetical point of view, and I expressed the belief that the megasporangial tapetum of the phanerogams, which in many

plants must be regarded as of great importance for conducting nourishment to the embryo sac, would likewise often be found to be hyperchromatic. This supposition has since been confirmed in the case of a single plant through the researches of Palm (1915) with *Dahlia coronata*, in the tapetum of which this writer observed normally occurring synkaryon-like nuclei with several nucleoli. Binucleate cells are, according to Palm, not found in the species investigated, and he states that an earlier observation, of which I had previously no knowledge — by Mdlle. Goldflus (1899) who asserts that binucleate megasporangio-tapetal cells are found in *Dahlia gracilis* — is doubtless incorrect. It might, however, be imagined that Mdlle. Goldflus' statement was correct as regards the species investigated by her. But her illustration (Pl. 1, Fig. 2) certainly shows only mononucleate elements.

The second point we will consider more closely. Němec (1910 and earlier) was led by his cytological experimental researches to the conviction that chloralized plant organs, e. g. root tips of *Pisum sativum*, obtained bi- or multinucleate, hyperchromatic cells, and that these cells, when the effect of the poison had passed off, underwent reduction division by autoregulatory means, whereby the normal chromosome number for somatic cells was restored.

The correctness of this result has been doubted by several writers. Kemp (1910) agrees, it is true, with Němec that the threads of the spindle are dissolved by the chloral hydrate solution, so that the daughter cells cannot separate, and multinucleate cells therefore arise, but in contrast to Němec, he states that such hyperchromatic cells do not participate in the active divisions, and that their presence can be discerned in the quiet undisturbed tissues higher up in the roots, some time after the poison action has ceased. He states that though his material of *Pisum sativum*, *Vicia faba* and *Galtonia*, does present pictures somewhat resembling those characteristic of the heterotypic divisions, they are in reality no indication of such.

And this is just where the difficulty lies; we are still uncertain what stages should be studied in order that the investigator can be perfectly sure that there is a heterotypic division.

Synapsis, which usually only takes place in gonokont nuclei prior to reduction division, has been observed by Strasburger (1904) in an apogamous *Alchimilla*, where the mentioned phase

was followed by an ordinary somatic division. — Diakinesis, which likewise generally denotes the prophase of the heterotypic division, has been noted by Juel (1904) in the apogamous *Taraxacum officinale*. The mother cell of the embryo sac divided only once, whereafter the one daughter cell degenerated, the other representing the embryo sac. The nucleus of this first went through synapsis, and then exhibited likewise a stage resembling diakinesis, but a revulsion then evidently took place, resulting in a mitotic division. Much the same was also observed by Strasburger in *Marsilia* (Roth 1907).

In all these cases we have throughout apogamous forms, in which, naturally, abnormal conditions may be expected to occur, and Juel's remark as to a revulsion taking place at the last moment in itself reveals as his view that the nuclei in question had prepared for a heterotypic division.

In the case of normal and normally sexed plants there are, as far as I am aware, only a very few records as to the occurrence of stages characterizing reduction division — apart from the regular appearance in the gonokonts.

Murbeck (1902) does mention diakinesis in the vegetative cells of *Ruppia rostellata*. The initial cells of the ovule form, according to the usual vegetative pattern, two daughter cells, the "tapetal cell" and the mother cell of the embryo sac respectively. One stage of this nuclear division Murbeck describes as "diakinesis" and he states that 16 chromosomes ($x = 8$) were here distinctly observed. The chromosomes do not, however, lie together in pairs, but singly, and the likeness to the diakinesis stage is therefore essentially restricted to the fact that the 16 chromosomes (Murbeck Pl. 2, Fig. 31) lie spread "diakinesis-like" throughout the nuclear membrane. As, however, the fact of the chromosomes lying in pairs is undoubtedly the most essential feature of the diakinesis, the writer's term for the stage observed seems to me somewhat ill-chosen.

Gates (1912) described a possible reduction division in the tissue of the nucellus in *Oenothera lata*. Here abt. 14 partly paired chromosomes are seen in the spindle.

In my work already quoted on the *Humulus* (1914) I stated that I had once, in a tapetal cell of an anther, observed a diakinesis-like prophase, where 16 to 17 chromosomes lay distributed throughout the nucleus in an absolutely diakinesis-like

manner. I illustrated this in my Pl. 1, Fig. 26, and was compelled to regard the phenomenon for the time being as abnormal. Since then, however, I have encountered it so frequentlly in the same species, *Humulus Japonicus*, that I am surprised that I should not have noticed it more often in my earlier studies. In reality, it would at least seem that the diploid nuclei in the tapetum and those of higher valency seem at any rate continually to go through the diakinesis stage in preparation for further divisions,

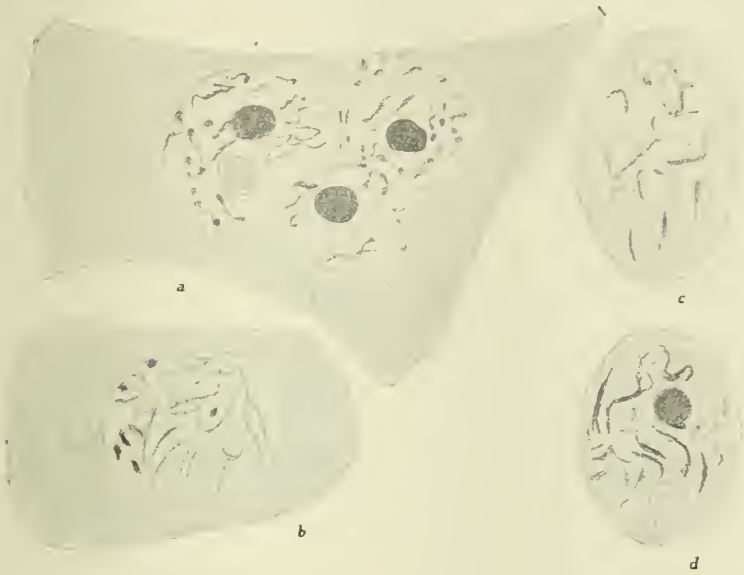


Fig. 40. *Humulus Japonicus*. Nuclei in the tapetum.
a, b, c, d respective stage of the prophase in nuclear division.

and only the brief duration of the phenonemon prevents it from occuring more frequently in fixed material.

I shall in the following endeavour to describe this method of nuclear division, which, owing to its peculiar course, cannot be compared with anything elsewhere in sporophytic tissue, but which is undoubtedly of a nature connected with the hyperchromatic qualities of the tapetal cells.

The process of nuclear division I presume — judging mainly from numerous notes and drawings — to take place as follows:

The earliest prophase (Fig. 40 *a—d*) is characterized by the fact that the chromatic substance appears at first in the form of long and thin, chromatin ribbons, which gradually become shorter and thus thicker, and appear in some degree, though not cons-

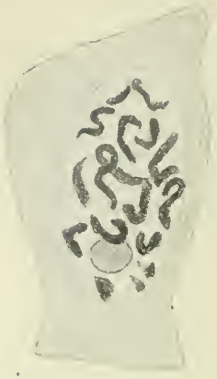


Fig. 41. *Humulus Japonicus.* Nucleus in the tapetum. Spireme stage

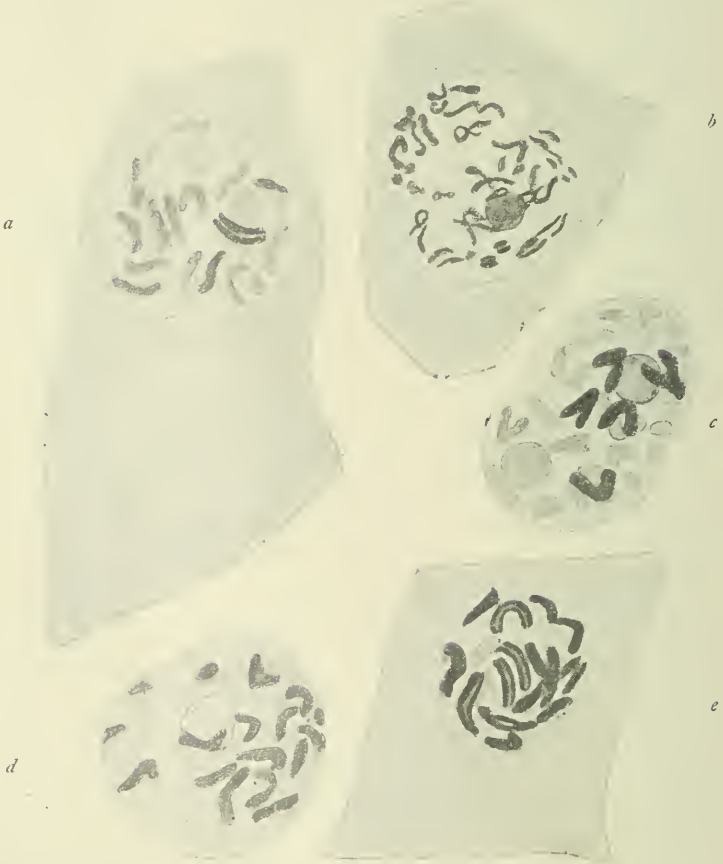


Fig. 42. *Humulus Japonicus.* Nucleus in the tapetum. Incipient longitudinal cleavage in the chromosomes.

picuously, to run in parallel pairs. This stage often considerably resembles synapsis. Fig. 41 indicates the following phase, a spireme stage, in which the chromatin ribbons have become a good deal thicker, and appear almost like chromosomes, each ribbon being of quite restricted length. They still twine, however, and curve in various directions. At this stage, I have only observed undivided chromatic units. The chromosomes then become further shortened, taking the form shown in Fig. 42, *a*—*e*

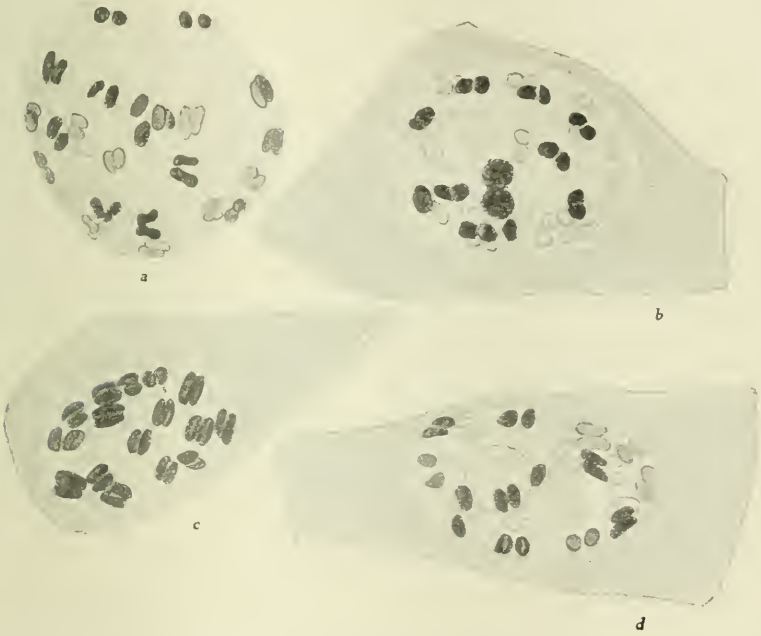


Fig. 43. *Humulus Japonicus*. Nuclei in the tapetum. Diakinesis-like stage.

from which also it will be seen, that longitudinal divisions here occur, highly suggestive of an approaching diakinesis. We find here precisely the same forms of chromosomes as in the stages preceding the diakinesis of gonokonts; gemini are seen, now lying parallel to each other, now Y- or U-shaped, attached only by the one end. — Fig. 43 *a*—*d* shows the process at its height, inasmuch as we have here a stage altogether diakinesis-like, save for the augmented number of chromosomes. The chromosomes lie in pairs in the periphery of the nuclear cavity. One or more nucleoli are present, which, by the way, have been visible all the time throughout the whole of the nuclear division process,

and the nuclear wall is still there, albeit extremely thin. — Naturally, the number of gemini depends upon the number and valency of the nuclei. Often two half-fused nuclei may have their division processes synchronously, and even, perhaps, in the course of the process fuse entirely together. On the other hand, two well separated nuclei in the same tapetal cell may at times be found at different stages. I have observed from abt. 20 pairs of chromosomes to at least 60 in each cell, i. e. x being = 10, from abt. 2x to abt. 6x gemini.

The chromosomes lying together are always of the same

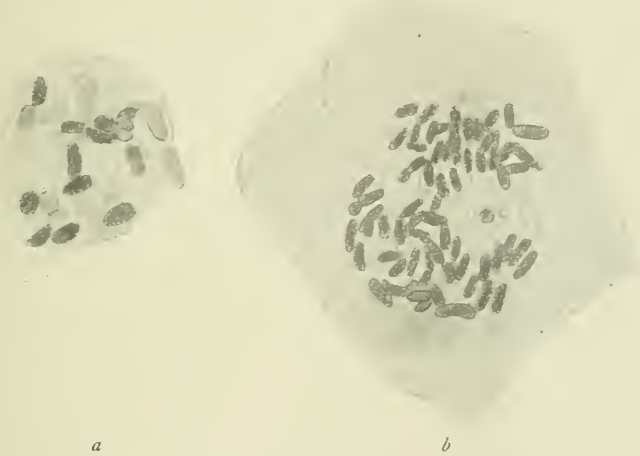
Fig. 44. *Humulus Japonicus.* Tapetal cell with nucleus. Gemini mostly separated.

shape, which bears witness to their common origin. I have now and again found that a pair of such chromosomes were distinctly split (Fig. 43 *c*) parallel with the plane of division. — In Fig. 44, the gemini are for the most part seen separated, only a few appearing still to lie together. In this figure, only the chromosomes in the upper half of the nucleus (or possibly a little more) have been drawn, so that there must have been a great number present; probably abt. 120 in all.

As will be seen, the paired chromosomes are separated already prior to the division of the nucleus itself. When this commences, after the spindle has been formed, the nucleoli and nuclear wall have disappeared, and the chromosomes are arranged

apparently in a very casual manner on the spindle (Fig. 45 *a—b*) and drawn towards the poles of the same, whereupon the daughter cells enter upon the resting stage.

What more especially will be found surprising in this nuclear division is the fact that splitting phenomena can apparently take place in the chromatic units at any stage. Even in the diakinesis stage, we can, as mentioned, at times observe a new split in the splitting products; this I have also noted in my previous paper (Pl. 1, Fig. 27). — Under consideration of reduction division in *Chelidonium* also, we noticed similar early splittings in the chromosomes, and altogether I must suppose that the chromosomes



a *b*

Fig. 45. *Humulus Japonicus*. Nuclei in the tapetum. Anaphase.

are so organized as generally to include split parts at various powers, in continual repetition.

If we now ask, whether it is possible that the processes just described really can denote a true reduction division, — such might be supposed, of course, to take place autoregulatively — the answer must absolutely be in the negative. — In the first place, according to my own experience as well as that of others, the nuclei of the tapetum become continually larger and richer in chromosomes during the developement of the anthers, which must be due to true chromosome divisions. In the second place, I cannot find any reasonable explanation why reduction divisions should take place in cells doomed to perish within a short time, and in which, moreover, reduction division would not have any

regulative effect at all, since no new cell walls are formed in the tapetum, so that reduction divisions would still have no effect upon the number of chromosomes. Furthermore, I have never observed any true synapsis.

The only explanation of the phenomenon — which is one of considerable theoretical interest — seems to me to be that we have here a special method of mitotic nuclear division, normally including — as regards the cytological view — a typical diakinesis stage, presumably due to anticipated chromosome splittings.

It is worth noting, that Strasburger (1908) mentions early chromosome splittings inter alia in *Lilium*, where, as we know, the chalazal nuclei of the embryo sac are hyperchromatic. He considers the splitting phenomena as connected with overnourishment of the nuclei, a suggestion which is excellently compatible with the occurrence of the mentioned splittings in the tapetum. A similar mode of nuclear division to that just described does not, however, appear to occur.

Fig. 46. *Atriplex littorale*. Nuclei in the tapetum. Diakinesis-like stage.

— It would be interesting to follow up this question further, also in the case of other plant species. I have myself observed exactly corresponding diakinesis-like stages in *Chenopodium album*. And in *Atriplex littorale* again, the nuclear divisions of the tapetum appear to proceed on similar lines, though I have not here observed quite so marked diakinesis stages (see Fig. 46).

Frisendahl (1912) mentions chromatin formation in the chalazal nuclei of *Myricaria Germanica* in the embryo sac — after reduction division — and Palm (1915) notes similar conditions in *Piper subpeltatum*, as also observed, by the way, in various other plants. The two mentioned writers have, in contrast to earlier investigators, described more or less closely the actual manner in which the propagation of the chromosomes should take place; they consider that the small grains of chromatin which appear during the resting stages, and which are present in far greater numbers than the haploid chromosomes,

differentiate themselves as such. Chromosome splittings on the other hand, they do not find, nor chromosomes lying in pairs. I shall not go further into the possibility that chromosome splittings such as I have just described might after all be supposed to occur, but will merely remark, that in the tapetal cells of *Humulus* also, we can discern views which, if certain stages only discoverable by seeking through a considerable number of cells should escape attention, might very well give the investigator the impression that a similar process to that described by Frisendahl and Palm took place.

Thus much then, we have ascertained with certainty: that the diakinesis stage — albeit with increased chromosome number — is not alone characteristic of the heterotypic division. And I consider it also beyond doubt, that prosynapsis and synapsis in such respect must be regarded as a better indication of approaching reduction; and, as far as I am aware, the only instances of synapsis not followed by a reduction division are those already noted in the case of some apogamous — i. e. abnormally sexed plant species.

In the present work, the nomenclature followed with respect to the plants which I have investigated is for Danish species in accordance with C. Raunkiær: Dansk Ekskursions-Flora, 3rd. Ed. by C. H. Ostenfeld and C. Raunkiær (Copenhagen 1914) and for others with the »Index Kewensis«.

All the cytological illustrations in the text, — with the exception of the microphotograph Fig. 7 — were drawn with the aid of Abbe's camera, using Zeiss' homog. Imm. 2 Mm. and Comp. Oc. 18. In the scale here given, they are magnified abt. 1900 diameters.

SUMMARY.

As the "cell" in several, especially primitive, organisms, e. g. amoeba, is without walls, and wall formation to be regarded as a morphologically and physiologically higher stage, which is in several plants connected with division of function, so also all would seem to indicate that the nucleus is of phylogenetically younger origin than the living cell itself, and that it indicates a physiologically greater differentiation within the same. — In lower organisms, such as for instance *Plasmodiophoraceæ* and *Entorrhiza*, the chromatin is not inseparably connected with the nucleus. In *Entorrhiza*, we even find in the spores a nucleus entirely free from chromatin, and free, split chromosome-like bodies in the cytoplasm.

A statistical survey of the haploid chromosome numbers in higher plants shows that the chromosome numbers can as a rule be resolved into the prime factors 2 and 3. More rarely, 5 and 7 appear as factors. From what is known, 8 and 12 must be regarded as the most frequently occurring chromosome numbers, and are far more often met with than the remainder: next in order come 16, and thereafter 6 and 9.

In the case of higher plants it is a rule that the species in a more or less systematical group have "related" chromosome numbers: i. e. values which are all simple multiples of one and the same cardinal number, and which enter into an arithmetical progression.

An investigation of 11 species — comprising 5 genera — of the family *Chenopodiaceæ* showed that all had 6, 9 or 18 chromosomes, whence the formula for *Chenopodiaceæ* would appear to be $x = 3n$ $(n \geqq 2)$.

Also in families which — like the *Compositæ* — are generally considered to be highly unstable with regard to the chromosome numbers, we find, on investigation of the separate subgroups, that a certain regularity is apparent. The chromosome numbers for the groups *Helianthеæ* and *Anthemideæ* can thus be characterized by $x = 8n$ and $x = 9n$ respectively.

In cases where it is desired to ascertain the possible relationship of less systematical units, the chromosome number will at times provide a last weighty argument pro or contra.

Apogamy, and the occurrence of double or manifold chromosome numbers, must be regarded as generally due to hybridization, whereby the chromosomes of the parent gametes are practically added together — but are paired only incompletely, if at all — in the zygote. It must be presumed that when chromosome pairing entirely fails to appear after hybrid fertilization, an indirect chromosome binding (see p. 199) must take place — if the zygote is to be capable of developement and propagation — whereby the number of chromosomes is doubled.

In *Callitriche verna*, the reduction division of the pollen mother cells gives rise to a kind of heterochromosomes, which cannot, however, be homologized with those found in the animal world. Corresponding phenomena have previously been observed in other plants, and especially hybrids.

The mottled-leaved Japanese hop, *Humulus Japonicus* var. *albomaculata*, affords an instance of the fact that certain qualities — here that of mottled colouring — are only transmitted to the offspring through the sexual cells of the female plant — and especially through the cytoplasm.

Cell- and nuclear divisions cannot proceed without an occasional loss of material, e. g. parts of chromosomes, which must affect the genotypic constitution of the cell.

It must be presumed that occasional hybridizations can give rise to the formation of apogamous, or at any rate sexually abnormal "new species", and that these newly formed biotypes and their offspring persist as special minor species or biotypes simply because they are, from their organization, excluded from fertilization, and thus from the adjusting — or mutually supplementing — effect which is, in the writer's opinion, produced by the alternation of generations.

Humulus lupulus can develope fruits containing a embryo on pollination with various related plants. Pollination with *Urtica urens* produced particularly large and strong fruits, though these were incapable of germination. It is presumed that a truly heterogeneous fertilization has taken place, and that the hybrid was capable of developement as long as it was nourished by the

mother plant, but that lack of internal homogeneity rendered it altogether incapable of independent activity.

In the hyperchromatic cells in the tapetum of phanerogams, nuclear division takes place according to a peculiar system, in which a typical diakinesis stage appears — albeit with doubled or manifold chromosome number; this does not, however, indicate any reduction division.

Copenhagen 23 June 1916.

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RACIAL INVESTIGATIONS.

UNDER the above common title it is proposed to collect from time to time the results arrived at by various studies of a general biological character, carried out by the Physiological Department of the Carlsberg Laboratory side by side with the more particular series: "Investigations on Hops", the results of which commenced to appear in 1913.

The aim of these racial investigations, which will be prosecuted chiefly in an experimental direction, is to elucidate, by means of suitable material, the less and least systematic units in natural history, and to study the constancy and inheritance of their qualities.

The first work, now to be published, is concerned, like several of the papers in the Hop series, with quantitative racial characters, but the subject has in this instance been drawn from the animal kingdom, from a species where the characters considered can be measured with a very high degree of accuracy.

Carlsberg Laboratory, 19th October 1916.

JOHS. SCHMIDT.

I. *ZOARCES VIVIPARUS* L. AND LOCAL RACES OF THE SAME

BY

JOHS. SCHMIDT.

With 3 charts and 34 figures in text.

I. Introduction.

AMONG vertebrates, the fishes are in several respects well suited for the purpose of racial investigations. In the first place, they present, in various important organs, certain numerical proportions which, while varying greatly in themselves, are easy to define and determine with great accuracy, an important point with investigations into racial characters. Such organs are for instance the vertebral column and the various fins, but many others may also be taken into consideration.

Another valuable point is the numerical frequency of individuals among fishes, and the consequent facility of procuring a material comprising many specimens from different localities. This is of course particularly the case when the species to be studied is a common article of trade. In such cases, it will be possible to procure, at comparatively slight expense, a mass of material far in excess of what could reasonably be obtained from among other vertebrates.

On these grounds, and also in view of the fact that several species of fish are in themselves economically important items, researches in this field have during recent years been keenly prosecuted, more especially since the publication of Heincke's classical investigations. This is evident from the numerous works wherein it is shown that marked racial differences do exist among several important species of fish species; I need only mention, by way of example, the herring and the plaice. The investigators

have succeeded, by purely descriptive methods, in discerning differences in several species of fish between the various "populations", "communities" or „stocks" inhabiting the different parts of the area covered by the species as a whole. Results of this nature may be of the greatest interest from the point of view of fishery biology and fishery statistics, but they tell us nothing as to the cause of the racial differences found. We are still unable to say, for instance, whether they are genotypically determined, or merely due to the influence of different external conditions in the environment of the various populations.

The fact is, that we have here to encounter very considerable difficulties, which render the fishes by no means always adapted to more exhaustive racial investigations. First of all, there are the experimental difficulties. Generally speaking, it is by no means easy to induce fish to propagate in captivity, or to rear the young when once obtained, and many species, as we know, take several years to arrive at sexual maturity. This applies at any rate to the majority of our principal northern food fishes, and we have here also to consider the further serious obstacle from an experimental point of view, that the eggs are fertilised and developed outside the body of the mother, often indeed pelagically.

There is, however, one species which in several respects constitutes a fortunate exception, to wit, the common viviparous blenny (*Zoarces viviparus* L.). This fish is not only viviparous, but also prolific, and is, moreover, not difficult to keep in captivity. It is thus possible to compare the qualities of the mother with those of a numerous progeny, as also to subject the parents during the time of pairing and gravidity to the influence of varying external conditions, in order to ascertain the effect produced upon the offspring.

It was by such considerations that I was induced, some years back, to commence a comprehensive study of the races of *Zoarces* in connection with experimental investigations supported by experiments with other species. The investigations are still far from completed, and in the present paper, I purpose for the most part to confine myself to the descriptive portion of the work, consisting of an exposition of the local races of *Zoarces* which have been found to exist in the Danish and adjacent waters.

I have on a previous occasion (1915, p. 18) stated the reasons

which led me, in a different connection, to select *Zoarces viviparus* for purposes of investigation. As indicated there, it was my original intention merely to utilise this species for purposes of comparison with the eel *(Anguilla vulgaris)* in order to show how great a difference might be found in racial respects between two species having more or less the same numerical features, but altogether unlike from a biological point of view.

The scope of this investigation, which was carried out for the International Investigation of the Sea, was thus very restricted, and its purpose soon attained. In course of the work, however, I was led to the conviction that *Zoarces* would probably prove suitable as material for the elucidation of several questions of a more general character with regard to the occurrence of local races.

I have therefore, at the Carlsberg Laboratory, resumed the *Zoarces* investigations, and continued and extended the work beyond the limits originally proposed. — During these further investigations at Carlsberg Laboratory, I have in various ways, — in the procuring of material, for instance — derived great advantage from being in close connection with the International Investigation of the Sea, and I am happy to have the opportunity here of acknowledging my indebtedness to that institution.

That *Zoarces* has been found in various ways well suited to the purpose of descriptive racial investigations is chiefly due to the fact of its being a highly localised species. And in this particular respect, there are certainly but few of our fish species which can surpass it. This is natural enough when we remember that it is viviparous, and has, practically speaking, no pelagic stage, such as is found among all our other fishes of economical importance, which are thus liable to be passively spread over extended areas. When the young *Zoarces* come into the world, they have already attained a considerable size, abt. 4—5 cm., and keep for the most part, like their parents, to the bottom. This fact, together with the presumable inability to make active migrations of any great extent is, I consider, an essential cause of the occurrence of numerous local races of *Zoarces*. which will be mentioned in the following.

The material on which the present work is based was drawn both from Danish and foreign waters, the former, however, as is natural, furnishing by far the greater part.

As regards these Danish waters, I have been able to pro-

cure material from almost all localities which I considered desirable. For this I have primarily to thank Fiskeriinspektør F. V. Mortensen, who kindly interested himself in the matter. The bulk of the Danish material has also been received through the agency of Fiskeriinspektør Mortensen's subordinates. I must especially mention Fiskerikontrollør L. Andersen, and in addition, also Fiskeribetjent R. Christiansen, Masnedø, and Fiskeribetjent A. S. Larsen, Mariager.

Valuable material has also been furnished from time to time by Hr. cand. mag. A. Otterstrøm, Snoghøj, and Hr. Proprietær N. Nielsen, Hov Vig, Nykjøbing Sjælland.

To several of my colleagues abroad I am indebted for material kindly furnished, especially Dr. T. Wemyss Fulton, Aberdeen, Professor E. Ehrenbaum, Hamburg, Dr. O. Nordqvist, Stockholm, Dr. H. C. Redeke, Helder, Holland, and Dr. K. A. Andersson, Fiskbäckskil, Sweden.

Constant or occasional participants in the execution of the work were, besides myself, Hr. cand. mag. Ths. Bartholin, Hr. mag. sc. Vilh. Ege, Frk. E. Fasting, Hr. G. Hansen, Hr. stud. mag. P. Jespersen, Hr. cand. mag. A. Strubberg, Hr. stud. mag. Å. Tåning and Hr. Dr. phil. Ö. Winge. The calculations were made by Frk. E. Schultz, Frk. cand. mag. Kirstine Smith, and Frk. E. Fasting.

To all these who have in various ways contributed to the progress af the present research, I beg to offer my best thanks.

Copenhagen, 19 October 1916.

II. Distribution and occurrence.

The distribution of *Zoarces* is in several respects remarkable, and therefore deserves some further notice.

The genus *Zoarces* is distinctly northern. In addition to the European species *(Zoarces viviparus)* there is a related one (*Zoarces anguillaris* Peck.) in the north of America, where, according to Jordan and Evermann (1898, p. 2457) it is found from Delaware to Labrador, and is fairly common north of Cape Cod. Finally, there is a third species described by Kner (1868, p. 344) *Zoarces elongatus* Kner, from the Sea of Okhotsk near the mouth of the Amur. Other forms than these are not known. As to the systematic value of the two latter, I can have no opinion, not having seen them myself[1]).

The European *Zoarces* is generally recorded as occurring from the White Sea in the north to Cadiz in the south, and from Ireland in the west to the Baltic in the east. These statements are, however, in part incorrect, or need, at any rate, to be carefully checked.

That it should be met with in the White Sea is apparent both from the statements of Smitt (1895. p. 607) and from the interesting work by Knipovitsch (1908) who has examined a large number of specimens from here, and has found the species

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[1]) The American *Z. anguillaris* has, according to Jordan and Evermann l. c., other numerical proportions than those which we know from the European species. Thus the number of rays in the dorsal fin is stated as 130 (95 + XVIII + 17) and for the anal 105, whereas in *Z. viviparus* the highest number of rays found in the dorsal is, as far as my knowledge goes, 125. It is possible, however, that a gradual scale of transition may be found to exist between the two forms. when a further number of American specimens have been examined.

With regard to the Asiatic *Z. elongatus*, this does not at any rate differ in numerical characters, as far as they are known, from *Z. viviparus*. According to Kner, the number of anal rays is 19—20, of dorsal rays 114 (80 + XII + 22) and of anal rays "over 90"; figures which are of very frequent occurrence in *Z. viviparus*. It is therefore possible that they may in reality be identical. See however postscriptum p. 396.

as far east as Tschesskaja Guba (abt. 68° N., 49° E.). According to Breitfuss' fishery experiments (1906) it should be fairly numerous on the Murman coast. It is also known on all the coasts of Norway (Collett 1875, p. 78).

Its southern limit of distribution appears to lie in the English Channel. Moreau (1881, p. 158) states that it is very rare on the French coast of the Channel, where it is only found at Crotoy, in the Bay of the Somme, and that it does not appear to move farther south. According to Day (1880—84, vol. I, p. 212) it should still be common as far west as the coast of Hampshire in the south of England. Dr. J. Allen, F. R. S., Director of the "Marine Biological Association's" Laboratory at Plymouth, informs me, in a letter dated $^{18}/_{11}$ 1914, that: "We very rarely, if ever, find a specimen of *Zoarces viviparus* here." In the comprehensive list of the fish fauna in the western part of the channel, which is given by Le Danois (1913) *Zoarces* in lacking. It therefore seems beyond doubt that a record in Steindachner (1868, p. 676) of its occurrence at Cadiz — a statement derived from Machado, and which has been dragged in everywhere throughout the literature — must be incorrect.

Though common on the east coasts of England and Scotland, *Zoarces* seems, strangely enough, to be lacking, or at any rate of rare occurrence, on the west coasts of Great Britain and Ireland. There is a record of ancient date (see for instance Day, l. c. p. 212) as to a find made in County Down, Ireland, but this has not since been confirmed. According to Thomas Scott (1900, p. 280), some few specimens have been taken in the Firth of Clyde, Loch Creran, Sound of Jura and Glenshiel, all places on or close off the west coast of Scotland. It does not appear to be common anywhere here. Herdman and Dawson (1902, p. 44) state with regard to its occurrence in the Irish Sea: "It seems commoner on the Scottish coast than here." . . . . "We have not yet found it in Lancashire, but it occurs on the Welsh coast." . . . . "Holyhead and Barmouth common" (Eyton); and Professor White tells us that he has seen "one or two specimens" at the Menai Strait.

Mr. E. W. L. Holt, Scientific Adviser to the Department of Agriculture etc. Fisheries Branch, Dublin, states in a letter of $^{31}/_{10}$ 1916: "I doubt whether *Zoarces* really exists on Irish coasts,

though there is an ancient and possibly erroneous record of it from the coast of County Down."

According to Day l. c. p. 212, *Zoarces* is common at the Orkneys and the Shetlands, but I have not myself seen any specimens from here.

In the east, it is found in the Cattegat and the Baltic, and penetrates here right up into the northernmost part of the Gulf of Bothnia (see for instance Lilljeborg, I, 1891, p. 538) and the eastern part of the Gulf of Finland (Kronstadt and mouth of the Narva, according to Knipovitsch 1908, p. 32).

Nowhere does the species seem to occur in such numbers as in the inner Danish waters and the adjacent Swedish and German; only here is it the object of industrial fishery to any extent. The waters in question, which may be taken together as the Western Baltic, can thus in a way be said to form the centre of the species' area of occurrence.

Zoarces exhibits a preference for shallow water, and the great majority of the specimens occur doubtless inside the 10 m. curve. It may, however, also go farther down, and I have some records from the Baltic (Bornholm) indicating that the species may here, at any rate during winter, be found at depths of 40—50 metres or more.

The Danish waters inside the Skaw, being shallow and rich in nourishment, thus also appear to particularly suitable as a habitat for the *Zoarces*, and the same applies to the Limfjord. On the other hand, it is less numerous on the North Sea coasts of Denmark, which is doubtless connected with the fact that these shores are less protected and poorer in nutritive matter.

Taking all the reliable data available as to the geographical distribution of the species, we come to the following results:

Zoarces is of distinctly north-easterly occurrence in Europe. From the White Sea in the north to the English Channel in the south it is regularly met with on the coasts of the European continent, as also on the east coast of Great Britain, and towards the east, it penetrates into the innermost parts of the Baltic.

On the west coast of Great Britain and round Ireland it is either lacking, or at the best of local and rare occurrence. As indicated in Chart Pl. 1, the western limit of regular occurrence appears to run from the Shetlands and Orkneys through Scotland and England to the northernmost part of the French coast.

Zoarces is not found at the Færoes or Iceland. This is very remarkable, since it differs here from practically all European marine fishes with a similar northerly distribution.

It will be natural to consider the non-occurrence or *Zoarces* in the waters named as connected with 1) the fact of its being distinctly a shallow-water form, and 2) the non-existence of any pelagic stage such as is found in most other species of fish, and which would enable it to traverse the great depths which separate Iceland and the Færoes from neighbouring countries. Bearing in mind the fact that it is found — or at any rate a related species occurs — on the east coast of North America, its non-occurrence at the Færoes and Iceland appears the more striking, as we can find no satisfactory reason why it should not be able to thrive there when it is met with, for instance, at the Shetlands and along the coast of Norway as far up as the White Sea. It is therefore most probable that the fact of its not being met with in Iceland and Færoe waters is rather due to geological and historical facts than to the present natural conditions presented by the grounds in question. This question, however, lies outside the scope of the present work, and I do not purpose to discuss it further here. I would merely mention that I hope, when occasion offers, to arrange for the liberation of *Zoarces* individuals in one of the fjords at the Færoes or Iceland in order to see how they thrive there, and if possible to follow the development of the stock under the new conditions.

III. Preparation of the material.

The samples investigated consisted as a rule of abt. 200 specimens each, this number being generally found suitable for reliable determination of the values for the characters concerned. The actual figures were, however, while sometimes in excess of this, also sometimes below it, as I was not always able to procure as many specimens from a certain locality as I had wished.

Altogether, the material upon which this present work is based comprises abt. 25,000 specimens, of which abt. 15,000 older stages and abt. 10,000 young (embryos).

The samples received for examination were treated as follows:

First of all they were sorted with regard to sex, distinction

being made between male and female specimens by cutting off with scissors a part of the gill cover from the left side in the female fish. The intestines were then removed, and the specimens placed in a 4 % formol solution, where they were left for some days, until well fixed. The formol was then washed out, and the musculature on the one side removed, exposing the vertebral column throughout its entire length.

Each specimen was given a serial number and placed in a bath of alizarine dissolved in alcohol, whereby the vertebral column, the fin rays, and other parts composed of bony tissue were stained red. As soon as the staining had taken place, the material was ready for examination.

In order to facilitate counting of the vertebræ, which number over 100, every tenth vertebra was marked with a spot of indian ink, a precaution which not only renders it easier to make the actual count, but also assists subsequent checking of the results. Similarly, in counting the fin rays, every tenth ray was marked off, in this case by splitting the fin.

The general rule in all these investigations was, that only figures to which no doubt attached were included: where any uncertainty prevailed, the specimen was discarded.

The following "characters" were determined:

1) Total length in cm (L). In several cases also, the age was determined by means of the scales and otoliths.
2) Sex (S).
3) Number of pigment spots on the dorsal fin at the right side from the fore edge of the fin to its incurvated part (Pigm D_1).
4) Number of vertebræ (Vert).
5) Number of rays in pectoral fins, as a rule in both (Pd and Ps), but at any rate in the right (Pd).
6) Total number of rays in the dorsal fin (D) and anal fin (A). These determinations were, however, soon restricted to certain definite samples, as it was found that the number of rays in these fins as a rule corresponds closely to (is correlated with) the number of vertebræ. (See for this p. 2— infra).
7) Number of rays in the caudal fin (C). For various reasons, these determinations were likewise restricted to certain definite samples.

8) Number of hard (spinous) rays in the dorsal fin (D_2). With exception of the samples first analysed, the determination of this character, to which my attention was first drawn by stud. mag. P. Jespersen, has been carried out for all specimens.
9) Number of soft rays in the posterior part of the dorsal fin (D_3). These determinations were only carried out in certain cases.
10) Number of branchiostegal rays (RBr). The determination of this character was soon relinquished, after I had found that in a large number of specimens (several hundred) no variation was observable, the figure being constantly six.

Of these characters, the following 4 four in particular have been utilised in the investigations: Number of vertebræ (Vert), number of rays in right pectoral fin (Pd), number of hard rays in dorsal fin (D_2) and number of pigment spots (Pigm D_1) in the anterior part of the dorsal fin. It is by the aid of these four characters that the determination of the different "races" of *Zoarces* in Danish and foreign waters has been made.

IV. Preliminary investigations.

As will be seen from the tables on p. 360, the proportion between number of males and number of female specimens varied greatly in the different samples; it was therefore necessary first of all to ascertain whether any difference existed between the sexes in regard to the characters concerned. For if such were the case, it would then be only possible to obtain representative and comparable samples by taking each sex separately, or by restricting the investigation to specimens of one sex only.

It was soon found, however, that no such difference — or at any rate no essential difference — existed between the males and females in respect of the characters in question. This will be apparent from the detailed information given in the tables of samples from different localities, p. 360, to which reference may be made. Here may also be noted the case of a sample from Vordingborg (S. coast of Sealand, Denmark, stat. 38), where males and females are considered separately, and from which it will be seen that the two sexes present the same appearance. From the

***Zoarces viviparus* from Vordingborg, March 1915.**

No. of vertebræ (Vert)	♂	♀	Sample No. 1 caught 15th March 1915	Sample Nr. 2 caught 24th March 1915
121		2	2	
120	2	5	1	6
119	14	9	12	11
118	15	30	11	34
117	25	24	26	23
116	42	55	41	56
115	35	38	28	45
114	15	25	13	27
113	11	9	6	14
112	2	3	3	2
111	1			1
n	162	200	143	219
a	**115.963**	**116.070**	**116.098**	**115.973**
σ	± 1.753	± 1.770	± 1.729	± 1.779
Fl.	± 0.464	± 0.421	± 0.487	± 0.405
No. of rays in right pectoral (Pd)				
21	1	2		3
20	54	58	44	68
19	104	133	88	149
18	15	16	11	20
17		3	1	2
n	174	212	144	242
a	**19.236**	**19.189**	**19.215**	**19.207**
σ	± 0.605	± 0.640	± 0.605	± 0.633
Fl.	± 0.155	± 0.148	± 0.170	± 0.137

same example also, it will be seen that two samples taken at different times likewise agreed (see further Section V, B, p. 294—298).

The characters which were found to be most easily applicable are then the number of vertebræ, number of hard rays in dorsal fin, number of pectoral fin rays, and number of pigment spots on dorsal fin. And as far as possible, a de-

termination of all these characters has been made in the case of all the samples dealt with.

At first, I also determined the number of rays in dorsal and anal fins. In view of the high figures involved, the work is here both troublesome and slow; nevertheless, the values were determined for several thousand individuals.

That I did not carry the determination of dorsal and anal rays throughout the whole of the material is due to the fact that as a rule nothing is thereby gained which can be regarded as essential in proportion to the work demanded. As will be seen from the figures below, the number of rays in dorsal and anal fins answers very closely to that of the vertebræ, or the caudal vertebræ; consequently, when the number of vertebræ has been determined, nothing essential is added by a further determination of the number of rays in dorsal and anal fins.

A sample consisting of 179 specimens with an average number of vertebræ 111.101 and a range of variation 108—114 gave the following results. All specimens in the sample having the figure for number of vertebræ 114 were examined for average number of dorsal and anal rays, and similarly with the specimens having 113, 112 etc.

Vertebræ (Vert)	Dorsal rays (D) (Average)	Anal rays (A) (Average)
114	112.5	90.5
113	111.2	88.9
112	110.4	87.8
111	109.4	86.8
110	108.4	86.0
109	108.0	85.2
108	106.5	85.0

These figures then, show that the number of dorsal and anal rays in this sample corresponded very closely to the number of vertebræ.

A similar result is obtained when we compare several samples having different values for average number of vertebræ, as will be seen from the following table:

	Vertebræ (Vert) (Average)	Dorsal rays (D) (Average)	Anal rays (A) (Average)
Sample No. 1 94 specimens	117.83	116.73	93.37
Sample No. 2 211 specimens	112.36	111.41	88.08
Sample No. 3 149 specimens	109.48	107.99	85.97

Furthermore, the coefficient (r) for correlation between number of vertebræ (Vert) and anal rays (A) was calculated for a sample of *Zoarces* from St. 31 in the Ise Fjord, consisting of 103 specimens. The result was as follows:

Vert (average)	113.54	A (average)	89.52
σ_{vert}	1.83	σ_A	1.86
Vert ÷ A.....	24.02	$\sigma_{vert \div A}$	0.86
r (coefficient of correlation)	+0.891	Probable error in r ...	+0.014

The calculation shows, then, that there is a marked positive correlation between the number of vertebræ and number of anal fin rays.

The correspondance between number of vertebræ and of rays in dorsal (and anal) fin is, by the way, a natural consequence of the fact that each vertebra (or caudal vertebra) — as a rule, however, with exception of the first or the first few — has a corresponding interspinous ray, borne on the upper (or lower) arch of the vertebra, and itself again bearing a ray. And it will also be seen from the figures given above, that the number of dorsal fin rays is on an average abt. 1 less than the average number of vertebræ, while similarly, the number of anal fin rays averages abt. 1 less than the average number of caudal vertebræ, i. e. rays with closed lower arches.

For these reasons therefore. the determination of number of rays in dorsal and anal fins was only carried out in certain special cases, where the point was of peculiar importance, and the figures are accordlngly not included in the tables for population analyses p. 360 *et seq.*

The number of caudal fin rays (C) was determined in the case of a number of samples, of which some instances are cited below:

No. of caudal rays (C).

No. of caudal rays (C)	No. of specimens in 5 samples (a—e)				
	a	b	c	d	e
14	1				
13	6		2		
12	19	22	18	1	7
11	39	69	153	206	100
10		3	1	1	17
9					3
n	65	94	174	208	127
a	**11.523**	**11.202**	**11.121**	**11.000**	**10.874**
σ	$\pm$ 0.734	$\pm$ 0.478	$\pm$ 0.376	$\pm$ 0.100	$\pm$ 0.525
Fl.	$\pm$ 0.307	$\pm$ 0 166	$\pm$ 0.096	$\pm$ 0.023	$\pm$ 0.157

The figures show that slight differences are discernible, but the differences are not only small; what is worse, the investigation here may at times present considerable technical difficulties, owing to the fact that it is not easy to determine in all cases whether a ray is to be regarded as the first in the caudal fin or the last in dorsal and anal fin.

I have therefore refrained from making determination throughout of the number of caudal fin rays in the samples examined.

I will now proceed to deal with the characters, which were, save in some few cases, determined for all samples, and comprise the number of 1) vertebræ (Vert), 2) rays in right pectoral fin (Pd), 3) hard rays in dorsal fin (D_2), and 4) pigment spots on dorsal fin (Pigm D_1).

V. Population analyses.

A. Population samples and composition of same.

In Tables p. 360 *et seq.* and on the charts will be found a detailed statement as to the investigations of the four characters in 61 samples of *Zoarces* stocks or populations.

As will be seen from the charts Pl. I—II, the samples are well distributed throughout large parts of the whole area of dis-

tribution of the species. There are, however, several places from which I have unfortunately been unable to procure material. In the case of Denmark, this applies for instance to the northernmost part of Jutland, on both sides of the Skaw, more especially the Skager Rack coast, where, however, *Zoarces* undoubtedly occurs but sparsely. Outside Denmark, there are naturally several places which it would be desirable to have represented, such as the innermost parts of the Baltic, with ramifications, the White Sea, the Shetlands and Orkneys, the southern part of the East coast of England, and the English Channel.

The majority of the population samples consisted of large specimens taken in traps or in eel seines. As a rule, the samples contained fish of several year-classes, as might be seen from counting the rings in the otoliths and scales, which offer a key to the age of the fish. Generally speaking, we may say that the three-year old fish made up the great bulk of the samples, though two- and four-year-olds were well represented. Five-year and one-year fish, on the other hand, were as a rule far less numerous. It should be noted however, that the age distribution of fish in a sample is chiefly dependent upon the implement of capture. A fine-meshed seine, for instance, will yield principally fish of the youngest year-classes, whereas by fishing with the hook, or laying out wide-meshed traps, the catch is likely to be restricted to the larger and older fish. We believe to have found specimens up to an age of nine years, determined with certainty.

It is naturally of great importance that the samples analysed should actually be representative of the populations in question; i. e. that the small section from the population of the place, which the sample consists of, really gives a picture of, "represents." That this may be the case, it is necessary that the number of individuals examined be not too small, though this will again depend upon the degree of accuracy with which it is desired to calculate the average of the population in any given case. As a matter of fact, a few specimens will often suffice to show that one population is essentially different from another; this will at once be seen from many of the analyses given in the tables (p. 360 *et seq.*) and on the charts. In every population analysis here carried out, we have calculated — according to the method employed in biometrical investigations — the probable fluctuation

of average[1]). By so doing, we obtain an idea as to the degree of accuracy with which the average value for the character concerned has been determined. For instance, supposing that we have in a population sample of 250 specimens found the average number of vertebræ to be 116.252, and have calculated that the fluctuation is ± 0.404, it follows that the true average for the number of vertebræ in that population will, with a probability of 1300—1400 to 1 lie within the limits of 116.252 + 0.404 and 116.252 ÷ 0.404, i. e. between 116.656 and 115.848. Should this degree of accuracy in any particular case be found insufficient, we can then have recourse to the expedient of increasing the number of variates, or in other words, we can count the vertebræ in a greater number of specimens. The probable error, and consequently also the field within which the true average lies, will thus be reduced; the fluctuation of the average is restricted.

We can thus, by purely mathematical means, estimate the degree of accuracy with which the average character, in casu the number of vertebræ, has been determined for any population. In this way it will often be possible to determine with certainty whether two populations differ in respect of the character concerned or not.

B. Constancy of the race picture, viewed in the light of repeated analyses from the same population.

If it is important to determine that a difference exists between two populations, it may be no less imperative to convince oneself of the constancy of the race picture found in a population. I have therefore had samples constantly taken, at suitable intervals, from several *Zoarces* populations in various parts of the Danish waters, and then determined the average values of the different characters.

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[1]) cf. Johs. Schmidt (1913, p. 11). The probable fluctuation of average is there, as here, calculated by multiplying the probable error by 5. The following formulæ were employed:

σ ("standard deviation") $= \pm \sqrt{\frac{\Sigma a p^2}{n-1} - b^2}$

P.E.A. ("probable error of average") $= \pm \frac{\sigma}{\sqrt{n}} \cdot 0.67449$

P.F.A. ("probable fluctuation of average") $= \pm \frac{\sigma}{\sqrt{n}} \cdot 0.67449 \cdot 5$

These investigations have not yet been in progress for a sufficient number of years to permit of any definite statement; I may, however, with due reserve, give the results of the two most complete series carried out up to date. The two populations in question are these from Nakkehage in the Ise Fjord (Stat. 31) and Snoghøj, Little Belt (Stat. 22). They were selected for these investigations as being particularly suitable from the fact that we have here a special guarantee that the consignments of *Zoarces* specimens received from time to time for investigations really are taken at exactly the same spot. I need not enlarge upon the vital importance of this point in constancy investigations, but would like to emphasise that we are very greatly indebted to the gentlemen furnishing the material in question, to wit, Hr. Proprietær N. Nielsen, Hov Vig, Nykjøbing, Sjælland, and Hr. cand. mag. A. Otterstrøm, Snoghøj.

I will take first the results from Station 31, Nakkehage in the Ise Fjord (see special chart on Pl. II). We have from here population analyses of samples collected during autumn of the three years 1914, 1915 and 1916, and in each case the number of vertebræ (Vert), number of pectoral rays (Pd), number of hard rays and pigment spots in dorsal fin (D_2 and Pigm D_1) were counted, with the exception of 1914, in which year the pigment spots were not counted.

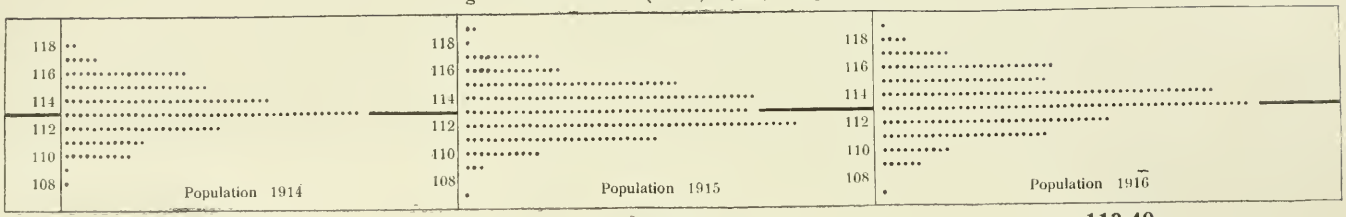
The counts are all shown as graphs, each character being taken separately. Each graph has a scale drawn on the vertical line, with figures showing the number of vertebræ, rays, etc. Each dot here represents, as also throughout the whole of this work, a specimen of *Zoarces* having the number of vertebræ, rays, etc. answering to the corresponding figure in the vertical scale. To facilitate reading, a thick black line has been drawn in the graph at a level with the variate nearest the average of the population. The average (a) and fluctuation (Fluct) are also noted in each graph.

The three first graphs (Fig. 1) show the number of vertebræ. We see that they are much alike, having about the same range of variation and about the same course. The average figures for the three years are 113.42, 113.23 and 113.40 respectively, and thus agree extremely well.

The three graphs in Fig. 4 indicate the number of rays in

Fig. 1—3. Stat. 31, Ise Fjord. Vertebræ (Vert), Hard rays (D_2), Pigment spots ($PigmD_1$) Constancy investigations.

Fig. 1. Vertebræ (Vert) 1914, 1915, 1916.

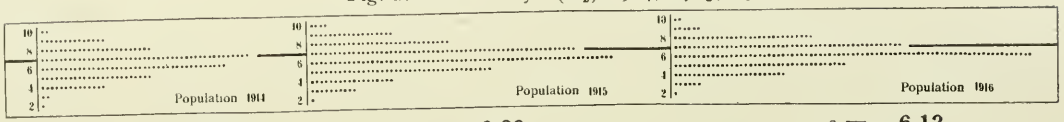


a = **113.42** Fl.= ± 0.49

a = **113.23** Fl.= ± 0.43

a = **113.40** Fl.= ± 0.44

Fig. 2. Hard rays (D_2) 1914, 1915, 1916.

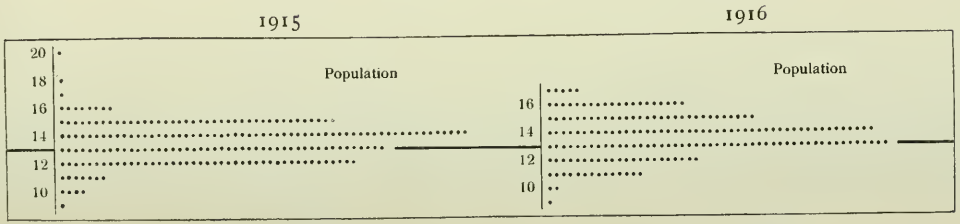


a = **6.49** Fl.= ± 0.39

a = **6.32** Fl.= ± 0.34

a = **6.12** Fl.= ± 0.31

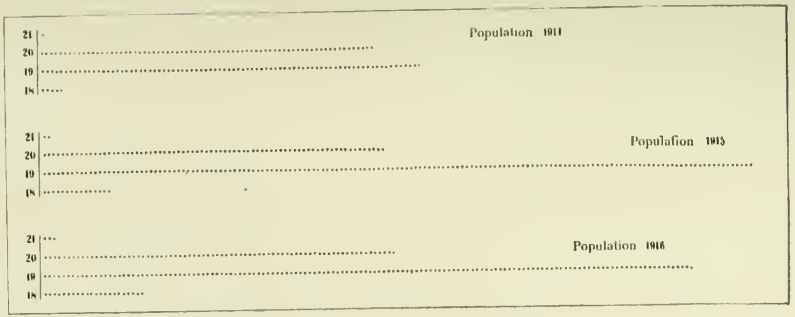
Fig. 3. Pigment spots ($PigmD_1$) 1915, 1916.



a = **13.50** Fl.= ± 0.34

a = **13.67** Fl.= ± 0.38

Fig. 4. Stat. 31, Ise Fjord. — Rays in right Pectoral (Pd). Constancy investigations.

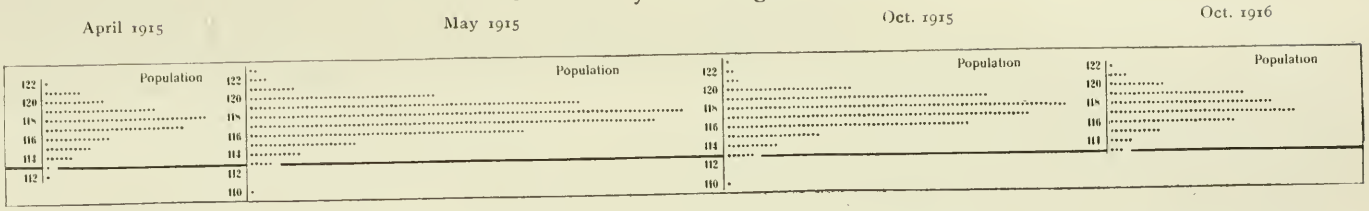


a (1914) = **19.43**. Fl. = ± 0.15

a (1915) = **19.26**. Fl. = ± 0.13

a (1916) = **19.26**. Fl. = ± 0.14

Fig. 5. Stat. 22, Little Belt. — Vertebræ (Vert).
Constancy investigations.



a = **117.69**
Fl. = ± 0.52

a = **117.65**
Fl. = ± 0.29

a = **117.44**
Fl. = ± 0.34

a = **117.44**
Fl. = ± 0.44

right pectoral. The averages for the three years are 19.43, 19.26 and 19.26.

We have then three graphs showing the number of hard rays in dorsal fin (Fig. 2). The yearly averages here are 6.49, 6.32 and 6.12.

Finally, the three graphs in Fig. 3 give the number of pigment spots for the years 1915 and 1916, with the averages 13.50 and 13.67.

The graphs next following (Fig. 5) represent population analyses from Snoghoj, Little Belt (Stat. 22), for April 1915, May 1915, October 1915 and October 1916. I need not here give the average figures for the different analyses, as they are noted in each figure.

Altogether, these repeated population analyses have revealed a considerable degree of constancy in the different characters. In no case was any certain difference found between the average figures for the population samples taken at different times.

It will, however, as already mentioned, be necessary to continue the investigations for a considerable time yet, before a conclusive decision can be claimed.

C. Can large areas be characterised by the qualities of their respective populations?

We may now proceed to consider more closely the result of the 61 population analyses carried out with the aid of material from widely different parts of the area of distribution of the species.

Detailed information will be found in the Tables 360 *et seq.*, where each character is noted separately. The various localities ("Stations") from which the population samples have been drawn have been numbered serially; the numbers will be found on the charts Pl. I and II, as also in the tables. Further decription of the localities, with statements of depth, position, etc. will be found in the list p. 351—359.

On the two charts (Pl. I and II) will be seen, besides the stations with their serial numbers, four figures noted for each station in different colours (red, black, green and blue). These figures indicate the average values for the different characters. Thus the number of vertebræ (Vert) is shown in red, the number of pigment spots ($PigmD_1$) in black, number of

hard rays (D_2) in green and of rays in right pectoral (Pd) in blue.

On glancing at the charts Pl. I and II, we find an extremely varied picture with regard to the combination of the four characters investigated. Thus a high red figure may occur together with a high blue (e. g. Stat. 22 Pl. II), or with a low blue (as at Stat. 53 Pl. I). A low red may also be found side by side with a high blue (Stat. 19 Pl. II) or with a low (Stat. 55 Pl. I). It may make matters easier if I explain at once that we have at many places in the shallow Danish fjords encountered a quite remarkably regular distribution or gradation of the characters. This distribution, which will be further referred to later on, we may for the present disregard, and merely consider the question as to how far the various areas may be characterised, in the rough, by the qualities of the *Zoarces* there found.

Omitting the Norwegian Sea[1]), from which we have not sufficient material, we may divide the sphere of occurrence of *Zoarces* into two parts: the North Sea area and the Baltic area, each of these again being subdivided into a western and an eastern portion, giving four areas in all: 1) The Western North Sea, 2) The Eastern North Sea, 3) The West Baltic area (including the Danish waters within the Skaw and the western Baltic) and 4) The East Baltic area.

For each of these four areas we take three stations, situated on the coasts outside the fjords, and calculate the mean of the averages for the three populations in respect of each character[2]). This gives us four mean values for each of the four areas, viz; number of vertebræ, pigment spots, hard rays and pectoral rays. These figures are arranged in groups in the chart, Pl. III, each

[1]) Knipovitsch, (1908) who has examined a large number of *Zoarces* from the White Sea and adjacent waters, does not give the number of vertebræ (and pigment spots), but for some few individuals the number of rays in P, A, D, D_2 and C. Judging from the figures given (l. c. p. 36—37) the form in question must be one with a high to medium number of vertebræ, medium number of pectoral rays and a high number of hard rays. In 12 specimens, the number of hard rays varied from 8 to 11, and averaged abt. 9,6. The *Zoarces*ca from the White Sea could accordingly seem to resemble mostly these of the Baltic areas.

[2]) The following stations were utilised for this survey: 1) Western North Sea Stat. 56, 57, 58. 2) Eastern North Sea Stat. 13, 54, 55. 3) West Baltic area Stat. 20, 22, 26. 4) East Baltic area Stat. 51, 52, 53.

group within its own area. In the accompanying table, the different characters are noted separately for each of the four areas, so that we can easily perceive their variation from west to east.

	Average value of	Vert	Pd	D_2	PigmD_1
I. North Sea area	1. Western part ..	116.2	18.71	7.2	12.7
	2. Eastern part...	111.2	18.48	6.0	12.3
II. Baltic area	3. Western part ..	117.7	19.35	8.0	14.3
	4. Eastern part...	117.2	18.66	11.1	12.3

As a general result then, we find that the West Baltic area, where the species occurs in greatest numbers, is characterised by 1) great number of vertebræ, 2) great number of pigment spots. 3) fairly great number of hard rays, and 4) great number of pectoral rays.

The East Baltic area has likewise a great number of vertebræ, but a comparatively small number of pigment spots, and pectoral rays, and again a very great number of hard rays.

The Western North Sea area resembles in three characters mostly the East Baltic, the number of vertebræ being great, or fairly great, and the number of pigment spots and pectoral rays comparatively small; it differs markedly, however, in having a comparatively small number of hard rays.

Finally, we have the Eastern North Sea area, which resembles mostly the Western North Sea area, in having a small number of pigment spots, pectoral rays and hard rays, though distinctly smaller even than there, while on the other hand it differs considerably from all other areas in having a very small number of vertebræ.

There can thus be no question of any regular rise or fall of the average values for the different characters as from west to east. It would indeed rather seem that the West Baltic area had the highest average values, these then decreasing both to east and west. This applies both to pigment spots and pectoral rays, but not to the number of vertebræ. This falls, it is true, towards the west, but not towards the east, and as regards the

number of hard rays, this follows a contrary course, rising markedly towards the east, and falling towards the west.

Altogether then, the results of this attempt at characterising the various divisions of the *Zoarces* area according to average structure of the respective populations may be summed up as follows: The manner in which the average values for the four characters investigated combine varies for the different areas. To a certain degree therefore, the combinations found may be said to characterise the areas concerned, at any rate in the rough, but there is no question of any regular gradation of the characters in relation to the geographical distribution.

We have now seen the main features in the distribution of the characters among the different areas, as illustrated by examples which may presumably taken as typical. On going into details, we meet with numerous exceptions and irregularities, which are often extremely difficult to explain.

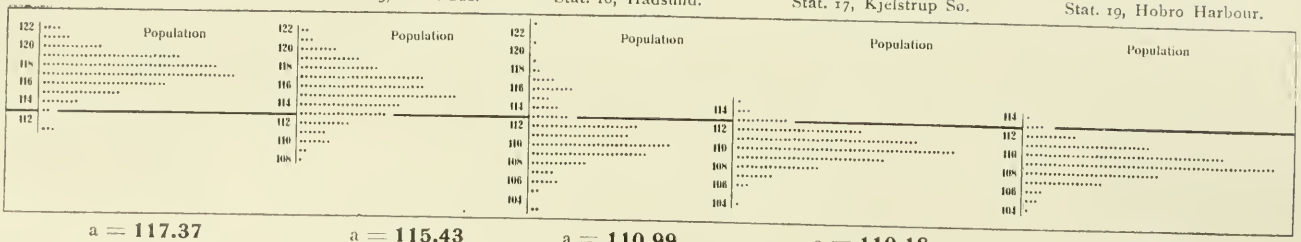
D. The Fjord populations.

In the shallow fjords of Denmark and Slesvig, highly peculiar conditions are encountered. By way of example, we may take Mariager Fjord on the east coast of Jutland, which has been well investigated, and of which a special chart on a large scale is shown in Pl. II. The results of population analyses from the different stations in Mariager Fjord will also be found in the graphs Figs. 6—9. Counting from outside up into the fjord, the stations are as follows: Stat. 14, Hurup, on the coast of the Cattegat, north of the fjord, Stat. 15, Als Odde, at the mouth of the fjord, Stat. 16, Hadsund, at the outer end of the narrow part of the fjord, St. 17, Kjelstrup Sø, a lagoon with narrow entrance channel on the north side of the inner part of the fjord, and St. 19, Hobro Harbour, at the base of the fjord.

A glance at the charts and at the graphs at once shows that there is a very distinct geographical distribution of the four characters concerned. Passing from outside up the fjord, we find the following average values:

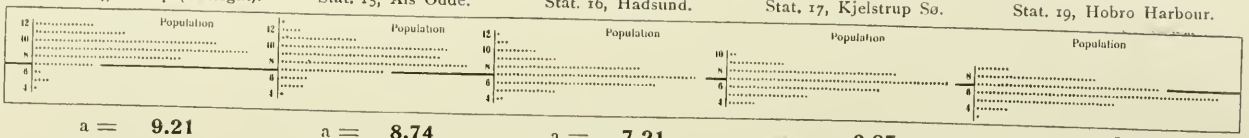
Fig. 6—8. Stat. 14, 15, 16, 17, 19, Mariager Fjord, Vertebræ (Vert), Hard rays (D_2), Pigment spots ($PigmD_1$). Showing decrease in average figures from mouth of fjord inwards.

Fig. 6. Vertebræ (Vert).



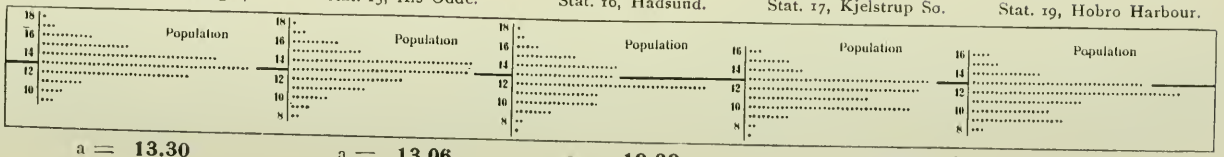
Stat. 14, Hurup (Cattegat).	Stat. 15, Als Odde.	Stat. 16, Hadsund.	Stat. 17, Kjelstrup So.	Stat. 19, Hobro Harbour.
a = **117.37**	a = **115.43**	a = **110.99**	a = **110.18**	a = **109.30**
Fl.= ± 0.48	Fl.= ± 0.65	Fl.= ± 0.84	Fl.= ± 0.43	Fl.= ± 0.40

Fig. 7. Hard rays (D_2).



Stat. 14, Hurup (Cattegat).	Stat. 15, Als Odde.	Stat. 16, Hadsund.	Stat. 17, Kjelstrup Sø.	Stat. 19, Hobro Harbour.
a = **9.21**	a = **8.74**	a = **7.21**	a = **6.87**	a = **6.40**
Fl.= ± 0.37	Fl.= ± 0.39	Fl.= ± 0.38	Fl.= ± 0.33	Fl.= ± 0.30

Fig. 8. Pigment spots ($PigmD_1$).



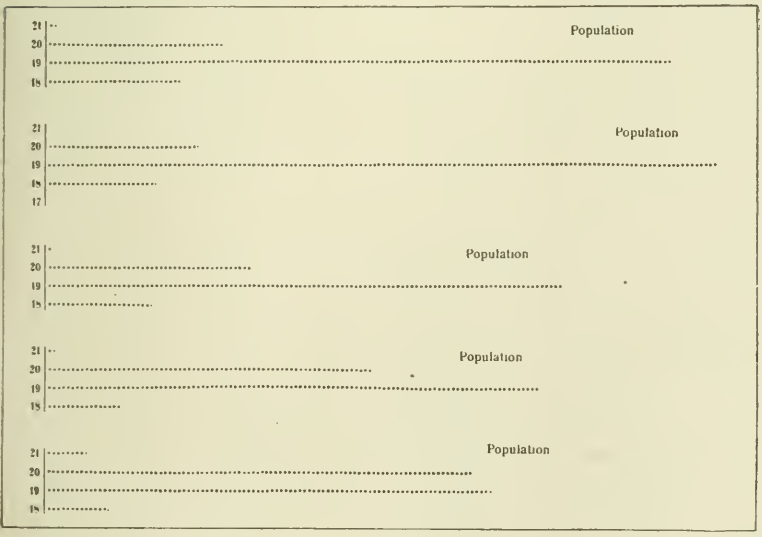
Stat. 14, Hurup (Cattegat).	Stat. 15, Als Odde.	Stat. 16, Hadsund.	Stat. 17, Kjelstrup So.	Stat. 19, Hobro Harbour.
a = **13.30**	a = **13.06**	a = **12.32**	a = **11.80**	a = **11.83**
Fl.= ± 0.42	Fl.= ± 0.49	Fl.= ± 0.54	Fl.= ± 0.44	Fl.= ± 0.46

Mariager Fjord:

Average value of	Vert	Pd	D_2	PigmD_1
Stat. 14	117.37	19.06	9.21	13.30
» 15	115.43	19.04	8.74	13.06
» 16	110.99	19.14	7.21	12.32
« 17	110.18	19.30	6.87	11.80
» 19	109.30	19.46	6.40	11.83

It will be seen from the table that the average number of vertebræ, hard rays and pigment spots falls as we pass inwards, whereas the reverse is the case with the pectoral rays, which have their highest mean value at the base of the fjord.

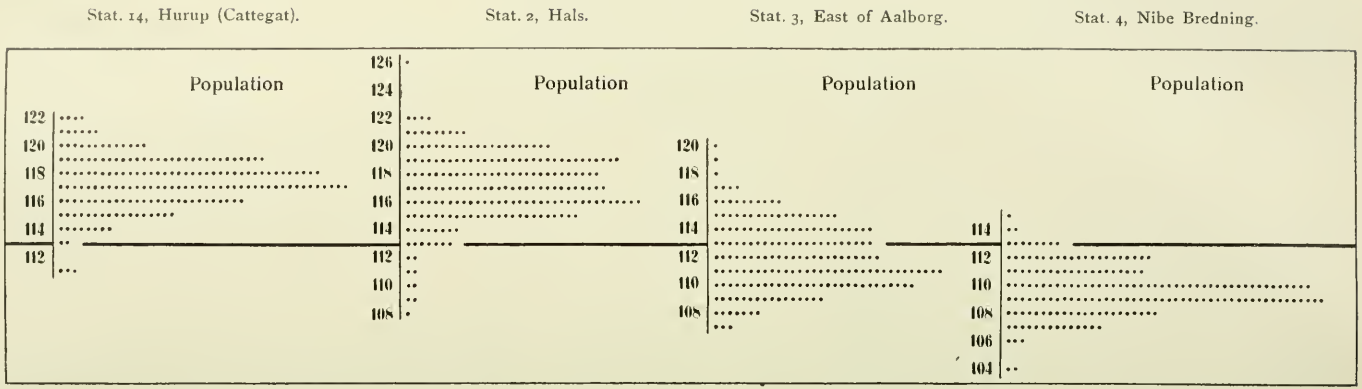
Fig. 9. Stat. 14, 15, 16, 17, 19, Mariager Fjord. — Rays in right pectoral (Pd).
Showing increase in no. of rays from mouth of fjord inwards.



A similar state of things is met with in the eastern part of the Limfjord (see chart Pl. II and graphs Fig. 10, as also the accompanying table).

Fig. 10. Stat. 14, 2, 3, 4, Eastern part of Limfjord, Vertebræ (Vert).

Showing decrease in no. of vertebræ from mouth of fjord inwards.



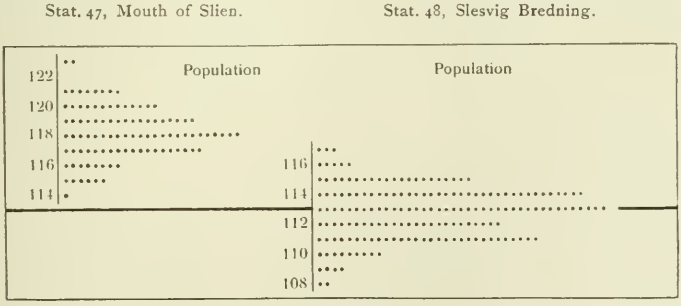
Stat. 14, Hurup (Cattegat).	Stat. 2, Hals.	Stat. 3, East of Aalborg.	Stat. 4, Nibe Bredning.
a = **117.37**	a = **117.10**	a = **112.11**	a = **109.69**
Fl.= ± 0.48	Fl.= ± 0.62	Fl.= ± 0.59	Fl.= ± 0.45

Eastern part of Limfjord:

Average value of	Vert	Pd	D_2	PigmD_1
Stat. 14	117.37	19.06	9.21	13.30
» 2	117.10	19.06	9.22	13.20
» 3	112.11	19.34	7.41	12.78
» 4	109.69	19.19	6.84	12.52

We find here precisely the same fall in the average values for vertebræ, hard rays and pigment spots from the Cattegat up into the fjord, while the mean value for pectoral rays also rises here, albeit not to so marked a degree as in the Mariager Fjord. And it should at once be pointed out that the conditions here indicated only apply to the eastern part of the Limfjord, as far as Nibe Bredning inclusive, i. e. only that part which really has the character of a fjord. Throughout the entire western portion, we find, as will be seen from the chart Pl. II, quite a different state of things, more resembling that seen in the North Sea, but this part of the Limfjord is, as we know, not properly of the nature of a fjord.

Fig. 11. Stat. 47, 48, Slien, Slesvig. — Vertebræ (Vert). Showing decrease in no. of vertebræ from mouth of fjord inwards.



a = **118.21** Fl.= ± 0.60

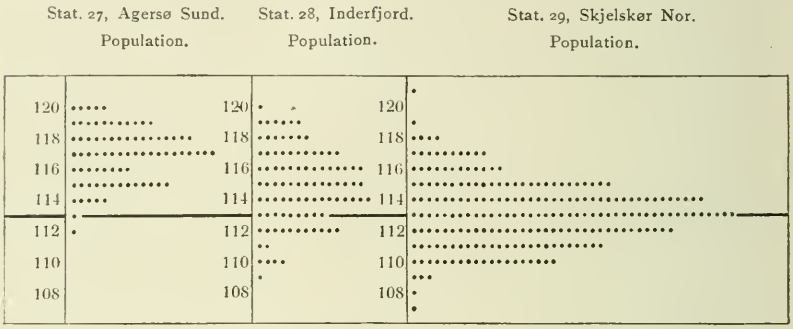
a = **112.81** Fl.= ± 0.45

Passing then to other fjords in the West Baltic area, from which investigations have been made, we find conditions similar to those noted for Mariager Fjord and the eastern part of the Limfjord.

Fig. 12—14. Stat. 27, 28, 29, Skjelskør Fjord. — Vertebræ (Vert), Hard rays (D_2), Pigment spots ($PigmD_1$).

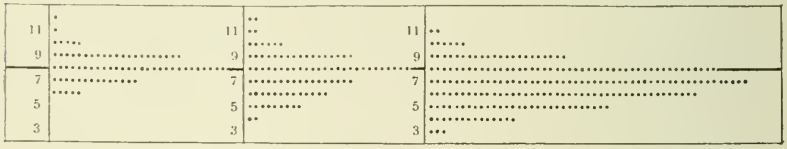
Showing decrease in average values from mouth of fjord inwards.

Fig. 12. — Vertebræ (Vert).



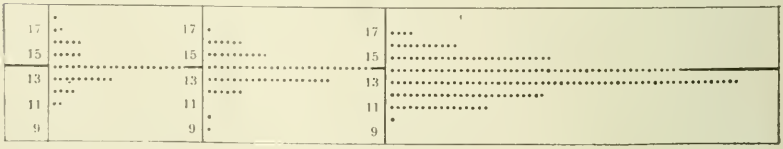
Stat. 27, Agersø Sund. Population.	Stat. 28, Inderfjord. Population.	Stat. 29, Skjelskør Nor. Population.
a = **116.94**	a = **114.85**	a = **113.17**
Fl.= ± 0.68	Fl.= ± 0.84	Fl.= ± 0.49

Fig. 13. — Hard rays (D_2).



St. 27, Agersø Sund. Population.	St. 28, Inderfjord. Population.	St. 29, Skjelskør Nor Population.
a = **8.19**	a = **7.63**	a = **6.82**
Fl.= ± 0.45	Fl.= ± 0.58	Fl.= ± 0.37

Fig. 14. — Pigment spots ($PigmD_1$).



St. 27, Agersø Sund. Population.	St. 28, Inderfjord. Population.	St. 29, Skjelskør Nor. Population.
a = **14.02**	a = **13.76**	a = **13.47**
Fl.= ± 0.69	Fl.= ± 0.54	Fl.= ± 0.35

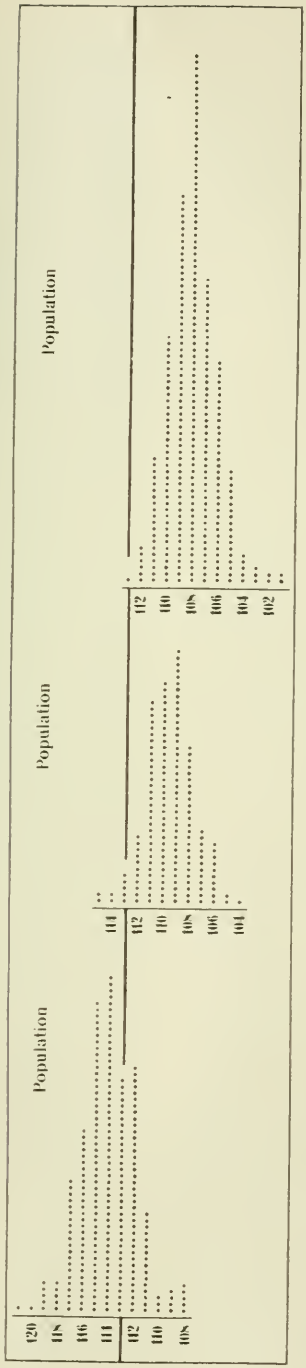


Fig. 15. Stat. 30, 33, 34, Ise Fjord—Roskilde Fjord. — Vertebræ (Vert). Showing decrease in no. of vertebræ from mouth of fjord inwards.

This applies for instance to Slien in Slesvig (St. 47 and 48), Odense Fjord (St. 25) in Funen, and Skjelskør Fjord, Sealand (St. 27, 28 and 29). Reference may here be made to the chart Pl. II and to the tables p. 360 *et seq.*, as also to the graphs Figs. 11—14.

The Ise Fjord, with Roskilde Fjord, north coast of Sealand, likewise affords nice examples, though we have not here examined material from the coast of the Cattegat outside the fjord. For Stations 30, 33 and 34, i. e. from the sea coast inwards, the mean values found were as follows. (See table p. 308, graphs Fig. 15 and special chart on Pl. II).

We here find, that the average number of vertebræ falls from 114 at the extreme outer part of the Ise Fjord to less than 108 in the innermost part of Roskilde Fjord, where the lowest average found anywhere was noted. Similarly also, the averages for hard

Ise Fjord—Roskilde Fjord:

Average value of	Vert	Pd	D_2	PigmD_1
Stat. 30	114.01	19.28	5.97	13.89
» 33	109.46	19.09	5.78	12.63
» 34	107.98	19.20	5.74	12.34

rays and pigment spots are seen to fall, while the number of pectoral rays does not exhibit any characteristic gradation.

As a general rule for the shallow fjords of the west Baltic area we may say, that the number of vertebræ, hard rays and pigment spots is smaller up in the fjord than at the mouth. As regards the number of pectoral rays, no definite rule can be stated, the value now rising, now sinking as we pass from the coast in towards the base of the fjord.

The differences noted as between the populations outside the fjords and these of the inner waters are distinctly discernible in the outward appearance of the fish. The *Zoarces* of the fjords are of markedly shorter and heavier build than those of the coast waters. Some idea of the difference in form may be obtained by comparing in two illustrations in Figs. 16 and 17, showing specimens with 121 and 105 vertebræ respectively. Apart from the difference in shape, the figures also show the dissimilarity in the number of pigment spots and of hard rays in the dorsal fin, the latter feature being apparent from the difference in size of the concave part of the fin.

The peculiar "fjord conditions" appear to become more marked in the larger and more well-defined fjord areas, but may nevertheless at times be met with in smaller fjords. We find for instance, that the average number of vertebræ falls from abt. 117.5 in the Little Belt to abt. 116.0 in Kolding Fjord (St. 23 Pl. II). In more open bays or creeks we may encounter exactly the same conditions as on the sea coasts. At St. 20 (Pl. II) in the innermost, northern part of Æbeltoft Vig, we find no decrease in the mean values for the characters in question, and the same would seem to apply, though in a lesser degree, to the populations of the open Nakskov Fjord (St. 42 Pl. II)

In the fjords of West Jutland, Ringkjøbing Fjord and Nissum

Fig. 16. Sketch of specimen 26.2 cm in length from Øresund, with 121 vertebræ, 12 hard rays and 16 pigment spots[1], in dorsal fin.

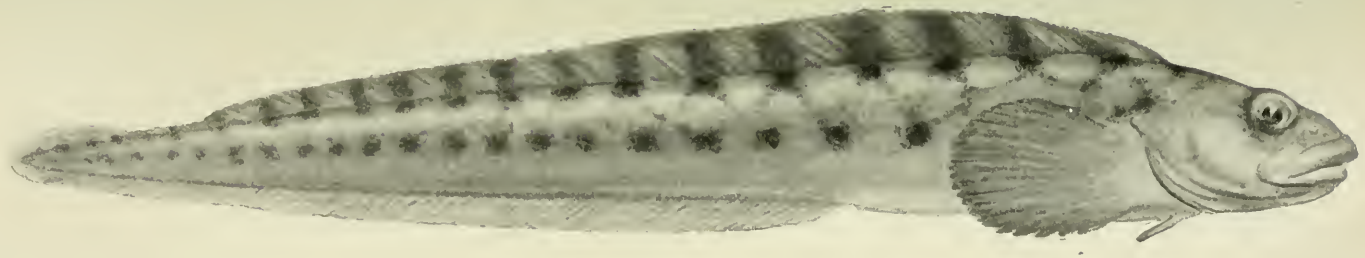
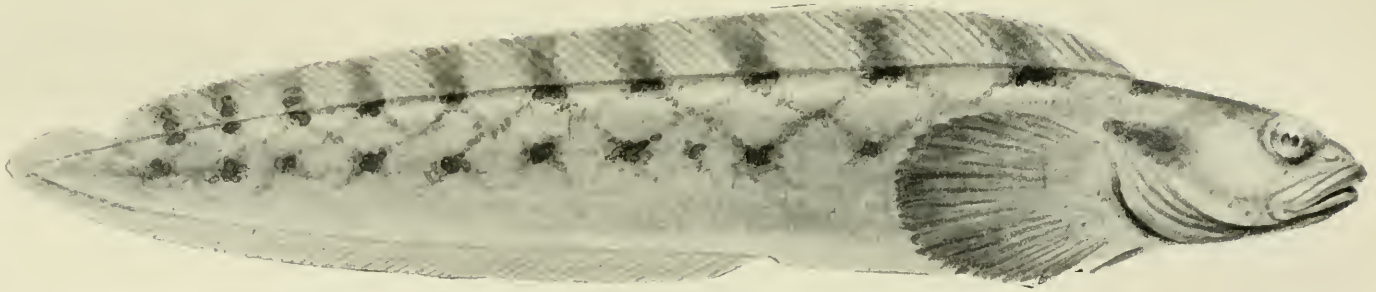


Fig. 17. Sketch of specimen 25.1 cm in length, from Roskilde Fjord, with 105 vertebræ, 5 hard rays and 11 pigment spots[1]) in dorsal fin.



[1]) The foremost pigment spot is situated on the anterior margin of the dorsal fin

Fjord (St. 10, 11, 12, Pl. II) we find similar low values for vertebræ, hard rays and pigment spots to those in the fjords inside the Skaw. Here, however, we are unable to make comparison between the inner and outer waters, having no material from the open coasts.

Outside the West Baltic Area, which is characterised by its shallow fjords with low shores, we have only population analyses from a few fjords. A good sample, however, is that from Drøbak, in the inner part of the Christiania Fjord (St. 61, Pl. I). As will also be seen from the chart, we found here the following average figures: Vertebræ 116.98, Pectoral rays 19.11, Hard rays 6.37, and Pigment spots 14.5. Here, then, we do not find the feature which most of all characterised the populations in the shallow Danish fjords, viz; the low number of vertebræ and consequent heavy build. On the contrary, the Drøbak population consists of elongated, slender fish. The number of pigment spots also is not low, but is on the contrary not far from the maximum value for this character[1]). We must then be content to note that in deep fjords on rocky coasts such as that of Norway, other rules appear to prevail than in the case of the shallow Danish fjords, which fact may be connected with the difference in natural conditions between Norwegian and Danish fjords.

E. Combination of characters in the different populations. Rules and exceptions.

On going through the results of the 61 population analyses with the aid of the charts Pl. I—II and the tables p. 360 *et seq.*, we find, as already mentioned, a marked variation in the manner in which the characters in question combine. This point I will deal with briefly here, and will for the sake of convenience disregard the pectoral rays, so that we have then only to consider the vertebræ, hard rays and pigment spots.

These are indicated either by *A*, *B*, and *C* or by *a*, *b*, and *c* respectively, *A* denoting a high, and *a* a low value for average

[1]) The average for hard rays, on the other hand, is low, only 6.37, and thus appears to correspond with what was found to be the rule for the Danish fjords. There is, however, no proof of this, as the number of hard rays may possibly be equally low in the populations living outside the Christiania Fjord, on the open coast of the Skager Rack.

number of vertebræ, while *B* and *b*, *C* and *c* are similarly used to represent high and low values for hard rays and pigment spots. *A B C* will then be a population with high average value for all three characters, *a b c* one where all three averages are low. The following eight combinations are possible:

A B C, *A B c*, *A b C*, *A b c*, *a B C*, *a B c*, *a b C* and *a b c*.

We will now proceed to examine which of these eight combinations were actually found in the population analyses made.

On going through the material, we find the following:

A B C (Stat. 42), *A B c* (Stat. 51), *A b C* (Stat. 61),
A b c (Stat. 56), *a b C* (Stat. 48), *a b c* (Stat. 34).

The two possible combinations *a B c* and *a B C*, however, are not represented, though the populations at St. 3 (possibly also Stat. 25) approach the *a B c*.

We find, then, six out of eight possible combinations realised, and it may perhaps be found on further investigations of populations from other localities that also the two here lacking exist elsewhere. At a certain stage of the investigations, I considered it certain that a high value for number of vertebræ was always combined with a high average figure for hard rays. We found that the two characters constantly appeared together, in the Baltic, where both were high, in the eastern North Sea, where both were low, and not least in the many Danish fjords, where both decreased gradually from the coast inwards. Then, however, we came upon the sample from Drøbak in the Christiania Fjord (Stat. 61), which showed that a high value for vertebræ, abt. 117 average, could be combined with a low number of hard rays, abt. 6.4 average, thus proving an exception to the rule.

Another example may be mentioned, this time with regard to the proportion between number of vertebræ and pigment spots. A high value for pigment (e. g. over 14 average) seems everywhere to be combined with a high value for vertebræ (see for instance St. 20, 22, 26, 27, 42 and 47, Chart Pl. II), until suddenly we meet with a sample from St. 48 in Slesvig Bredning, where the high average of 14.25 is seen combined with a figure for vertebræ as low as 112.8, and we are forced to admit that the rule which we believed to have found is not without exceptions, etc. etc.

Our observations as to combination of characters may be summed up as follows:

Fig. 18. Sketch of specimen, 32.8 cm long, from Gullmarsfjord, Sweden (Stat. 50), with 109 vertebræ. The dorsal fin lacks hard rays, and has thus no incurvation.

The characters investigated may be combined in very different ways, and the mosaic design formed by these combinations gives us the race picture. Not all the possible combinations have been found realised, and these met with are not all of like frequency. In many cases it has been found that a rule which it seemed justifiable to establish on the basis of numerous population analyses did not always hold good in reality.

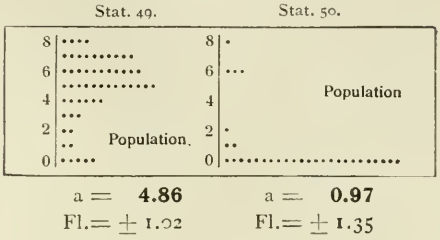
I do not propose to go farther into the results of the separate population analyses, but will refer the reader to the charts and tables. One particularly characteristic, however, may well deserve mention here.

A highly peculiar feature in the structure of the *Zoarces* is, as we know, the hard-rayed part of the dorsal fin, which makes itself apparent to the external view as a slight concavity in the hinder part of the fin. This incurvation may, as will be seen from the two sketches figs. 16 and 17, vary considerably in length, according as the number of hard rays is greater or less. The average number of hard rays varies from abt. $11^1/_2$ in the Baltic to abt. $5^1/_2$ in the southern part of the North Sea. We have, however, found a population on the west coast of Sweden off and in Gullmarsfjord, where a large num-

ber of specimens entirely lack hard rays, and have thus no concavity in the dorsal fin at all. A specimen of this type is shown in the sketch Fig. 18.

As will be seen from the two graphs Fig. 19, not all the specimens lack hard rays, but the average figure is here reduced to a value unknown elsewhere, to wit, 0.97 and 4.86 respectively. It should also be noted that the two graphs Fig. 19 present the only marked instances of bimoal curves, though of a peculiar nature, which we have found in the population analyses as a whole.

Fig. 19. Stat. 49 og 50, Gullmarsfjord, Hard rays (D_2).



F. Comparison between *Zoarces* and another species with similar numerical proportions (*Anguilla*).

We have seen in the foregoing, that the European *Zoarces* are distributed among a large number of local races, characterised by numerical proportions and distribution.

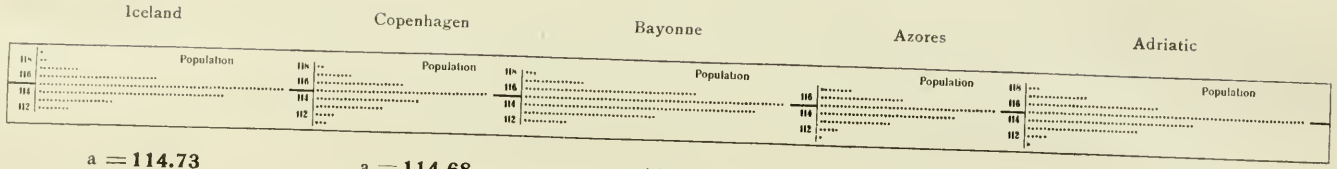
It will now be interesting for purposes of comparison to examine the racial picture presented by another species with similar numerical proportions to those of *Zoarces*. Such a species is the common eel (*Anguilla*). I therefore take some figures from previous publications (Johs. Schmidt, 1913 and 1915), which are here shown graphically in Figs. 20—22, in the same manner as the other figures used in the present work.

By means of a large number of population analyses made with material from many different places, I have shown that the common eel (*Anguilla vulgaris* Turt.) is found from the northern Norwegian waters southward along all the coasts of Europe and those of North and North-west Africa, as also at the Færoes and Iceland, the Azores, Madeira and the Canaries. The species is thus of far wider distribution than *Zoarces viviparus*.

In the population analyses, various characters were determined; I will here merely mention the number of vertebræ and number of pectoral rays. Fig. 20 shows graphically the number of vertebræ in 5 population samples from Iceland, Copen-

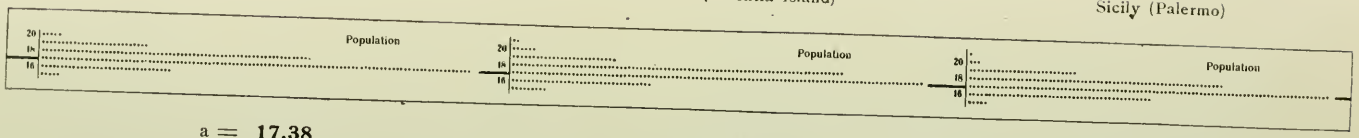
Fig. 20—21. The European eel (*Anguilla vulgaris*). Vertebræ (Vert) and rays in right pectoral fin (Pd). Showing that the populations are identical without regard to locality.

Fig. 20. — Vertebræ (Vert) in 5 populations.



Iceland	Copenhagen	Bayonne	Azores	Adriatic
a = **114.73**	a = **114.68**	a = **114.67**	a = **114.60**	a = **114.77**
Fl. = ± 0.32	Fl. = ± 0.30	Fl. = ± 0.21	Fl. = ± 0.38	Fl. = ± 0.31

Fig. 21. — Rays in right pectoral (Pd.) in 3 populations.



Iceland (Faxe Bay)	Ireland (Valentia Island)	Sicily (Palermo)
a = **17.38**	a = **17.41**	a = **17.32**
Fl. = ± 0.21	Fl. = ± 0.22	Fl. = ± 0.23

hagen, Bayonne, the Azores and Comacchio (Adriatic); i. e. from widely separated localities within the large area of distribution. From these graphs we see 1) that the same figures are found as in the case of *Zoarces*, and 2) that all the samples are identical. A similar result was arrived at on investigation of other characters. The graphs in Fig. 21 give the number of pectoral rays (right) in three populations from Iceland, Ireland, (Valentia) and Palermo (Sicily). Here also, we find complete identity, and the same applies to all other characters in the many population samples of eels examined.

The eel then, differs altogether from the *Zoarces*. Despite the fact that its area of distribution is far larger, we find throughout the same race.

The cause of this must, as I have previously (l. c. 1913) shown, be sought in the fact that the eel has a far greater power of migration than *Zoarces*, as indicated *inter alia* by the fact that all the eels of Europe migrate out into the Atlantic to breed there. The offspring — the larval stages — spend the first part of their life out there, and form during their ocean period the entire complement of vertebræ, fin rays, etc.

When therefore the elvers later appear on the coasts of Europe, they have their "racial cha-

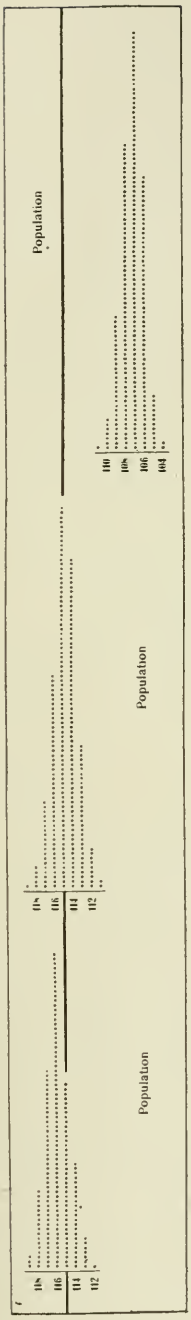


Fig. 22. — The Japanese, European and American Eels (*Anguilla japonica, vulgaris* og *rostrata*). — Vertebræ (Vert).

racters" fully developed, and the result must be that all eel populations, whether from Iceland, the Azores, the Baltic waters, or the shores of the Mediterranean, are identical; they belong to one and the same race.

But are there then no different races of eels, comparable with those found in the case of *Zoarces*? The answer to this question will be found in the graphs Fig. 22, showing the number of vertebræ in three eel populations from Denmark, The United States and Japan. We here see, that we must go far as America and Japan to find other races of eels; and even these moreover, do not differ more markedly from our European eels than do the *Zoarces* of the coast outside Mariager Fjord from those farther up at the base of the fjord.

A comparison of *Zoarces* with the eel is thus highly instructive. It shows how great a difference there may be between two species having the same numerical proportions and partly the same distribution, the one being divided into many highly restricted local races, the other belonging everywhere to one and the same race.

From this we see how great may be the importance of biological peculiarities in a species for the occurrence of local races.

VI. Individual offspring analyses.

In the foregoing section we have seen the result of a large number of population analyses, and thus also the geographical distribution of the various combinations of characters.

The purpose of this present first paper is as a matter of fact merely to set forth the more descriptive portion of the work with the *Zoarces* races, the experimental part being reserved for a later publication. Nevertheless I have thought it best to make some little reference in these papers to the results of the numerous "individual offspring analyses" which I have carried out, *inter alia* so as to furnish a broader basis for discussion as to the nature of the races.

That such individual offspring analyses are possible at all is due to the fact that *Zoarces* is a viviparous fish, and it is thus easier to work with in this respect than most other species of fish.

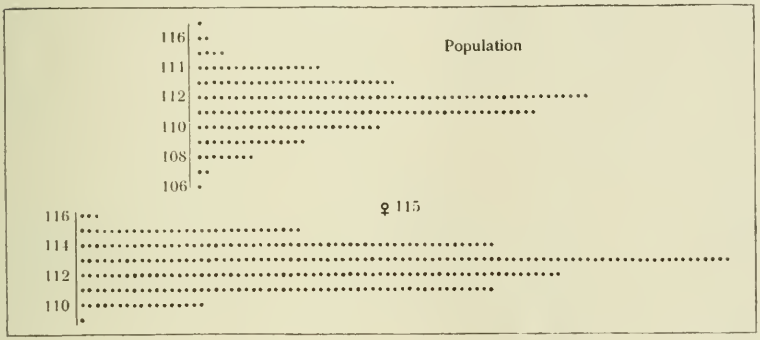
During the winter months, nearly all female specimens of

sufficient age and size contain large embryos[1]). The number of these may vary according to the age of the mother, and also according to the race, but is as a rule considerable, e. g. 50—100—200, or even 400 specimens.

It happens fortunately, that all the characters which we have measured in the population analyses are easily and certainly determinable in the larger embryos, either directly, without preparation (pigment) or after staining the bony tissue with alizarine and subsequently by clarifying with xylol (vertebræ and fin rays).

I may commence by taking an instance of the number of

Fig. 23. — Stat. 7, Limfjord. Vertebræ (Vert).
Population sample and individual offspring sample.



a (Pop.) = **111.49** a = **112.54**
Fl. (do.) = ± 0.42 Fl. = ± 0.26

vertebræ in one of the largest offspring samples examined. It was taken from a specimen with 115 vertebræ, caught in November 1914 in the Limfjord on the east coast of Mors (St. 7) and consisted of 300 specimens, a number thus permitting of exact determination. The result of the investigation is shown in the accompanying graph, Fig. 23, and for purposes of comparison, a graph showing the number of vertebræ in the population of the same locality is appended.

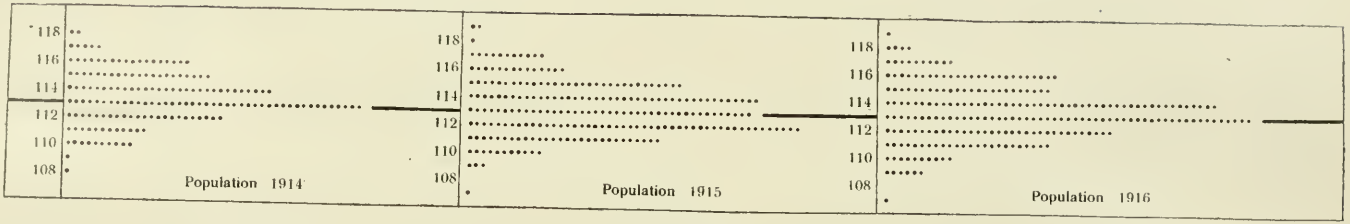
It will be seen that the offspring graph resembles the population graphs of which so many specimens have been considered in the foregoing.

[1]) *Zoarces* can produce offspring when 2 years of age. For development and growth of the embryos during the period of gravidity (Sept.-Febr.), detailed information will be found in Jungersen's important work (1889), where also older literature on the development of *Zoarces* is mentioned.

Fig. 24—25. — Stat. 31, Ise Fjord. — Vertebræ (Vert).

3 population samples and 3 individual offspring samples.

Fig. 24. — Population samples 1914, 1915, 1916.

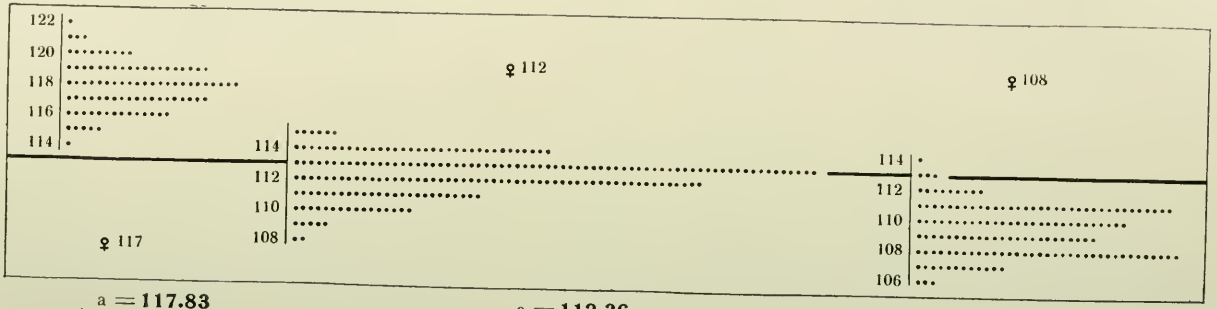


a = **113.42**
Fl. = ± 0.49

a = **113.23**
Fl. = ± 0.43

a = **113.40**
Fl. = ± 0.44

Fig. 25. — 3 individual offspring samples.



a = **117.83**
Fl. = ± 0.55

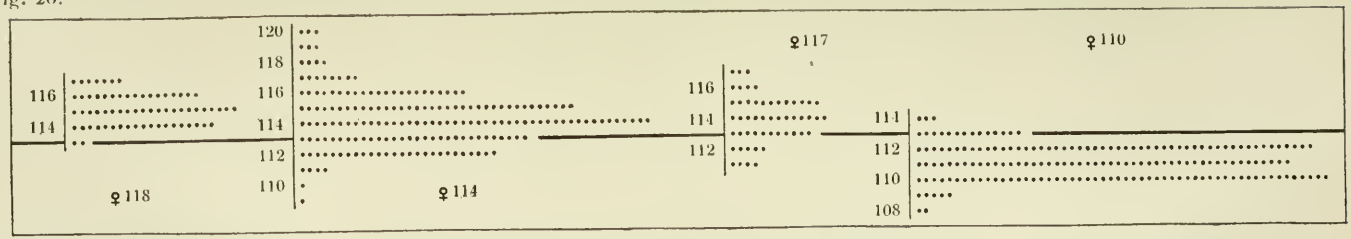
a = **112.36**
Fl. = ± 0.32

a = **109.48**
Fl. = ± 0.45

Fig. 26—27. Stat. 31, Ise Fjord. — Vertebræ (Vert).

8 individual offspring samples.

Fig. 26.



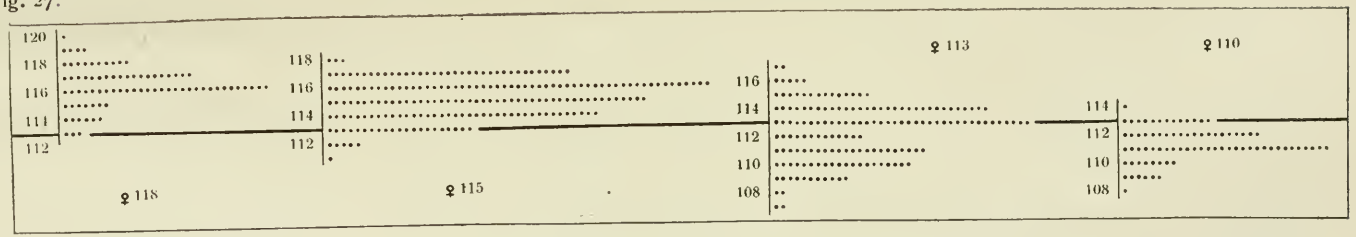
a = **115.12** Fl.— ± 0.43

a = **114.27** Fl.= ± 0.47

a = **113.92** Fl.= ± 0.72

a = **111.106** Fl.= ± 0.28

Fig. 27.



a = **116.34** Fl.= ± 0.54

a = **115.17** Fl.= ± 0.33

a = **112.37** Fl.= ± 0.55

a = **111.33** Fl.= ± 0.46

On comparison with the population graph, however, we notice one marked difference. Despite the fact that the number of specimens is greater than in the population graph, several of the variates found in the latter are here lacking, to wit, 106, 107, 108 and 117, or particularly the lowest figures. In addition, the mode is different, being at 113 and 112 respectively, and the same applies to the average, which is 112.54 and 111.49 respectively.

We find then, that the offspring sample is not identical with the population sample. The difference, however, in this case is not great, albeit the averages of the two samples do not "fluctuate together".

Let us now take another sample, turning this time to the Ise Fjord, from which a great mass of material is available.

The average number of vertebræ of the population lies, as will be seen from the graphs Fig. 24, for this locality (St. 31) between abt. 113.2 and 113.4, as indicated by investigations of 1914, 1915 and 1916. From the individual offspring analyses we may obtain entirely different figures. Examples of this will be seen from the graphs Figs. 25—28, p. 318—319.

Even these few examples reveal average values varying from 117.83 to 109.48, and can thus diverge very considerably from the population average. For purposes of comparison, the population graphs for 1914, 1915 and 1916 are shown in Fig. 24, where also a horizontal line has been drawn at a level with 113, the variate nearest the average of the population samples. This line is intended to facilitate comparison between the population samples and the offspring samples.

During the three years 1914—16, several hundred analyses of individual offspring samples were made with material from St. 31.

To analyse every single embryo from each particular female examined was out of the question here, the material being too extensive. I therefore took ten embryos (a "decade") from each specimen for examination ("decade investigations").

It would take too much space to give here the results of all these decade investigations, but the table p. 390, Section XII shows the average number of vertebræ for each decade for the year 1914, together with the fluctuation of the average.

It will be seen from the table that the average of the separate offspring samples varies from 118.9 to 109.3, which figures are

not far from representing the highest and lowest respectively of the averages found in all the *Zoarces* populations.

In other words, the small Nakkehage population (average abt. 113.2—113.4) contains individuals whose offspring in point of average number of vertebræ approach the highest, and others again nearing the lowest average noted for any population within the large area of distribution of *Zoarces*.

The relation between average of the population and that of the offspring is one side of the question, which may be elucidated by offspring analyses. The other side is the relation between the characters of the offspring and those of the mother.

In each of the offspring graphs Figs. 25—29, the figures for the mother are shown. The examples chosen show that the average in an offspring sample may lie very close to the figures for the mother, but may also differ considerably from the same, either in "positive" or "negative" direction. This point, however, I will not discuss further at present, as it will be further dealt with on a subsequent occasion.

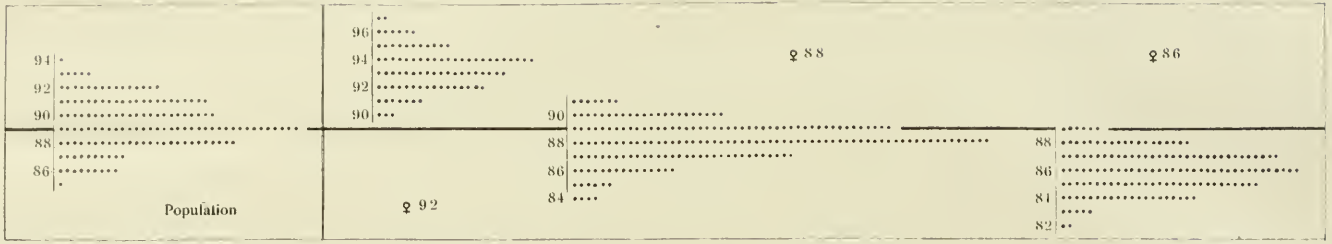
What we have noted hitherto applies only to the vertebræ for the population from St. 31 in the Ise Fjord, but entirely similar conditions are found among many other populations. I will here merely mention a couple of examples. They are taken from the populations at St. 22 in Little Belt and St. 33 in Roskilde Fjord, where the average values for vertebræ are abt. 117.5 and abt. 109.5 respectively. In tables p. 391—392, Section XII, the average values for a series of individual offspring analyses (decades) are shown in the same manner as for St. 31 in the Ise Fjord. On going through these figures, we find that the Little Belt population contained individual offspring samples whose average varied from 120.3 to 113.3, while the Roskilde Fjord population had samples varying from 115.9 to 106.6. It was thus soon found, by these few analyses of the elements in the two populations, that the first contained individuals producing offspring with a higher, the other individuals whose offspring had a lower average number of vertebræ than found in any population at all.

The number of vertebræ is not the only character which presents the features above described. This will be seen from some examples, taken chiefly from the frequently mentioned population at St. 31 in the Ise Fjord.

There are for instance the number of rays in the anal fin

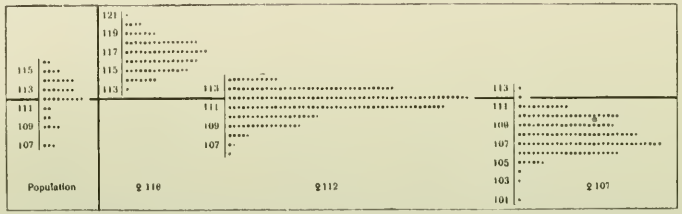
Fig. 28—30. Stat. 31, Ise Fjord. — Anal fin rays (A), Dorsal fin rays (D), Rays in right pectoral (Pd). Population samples and individual offspring samples.

Fig. 28. Anal rays (A).



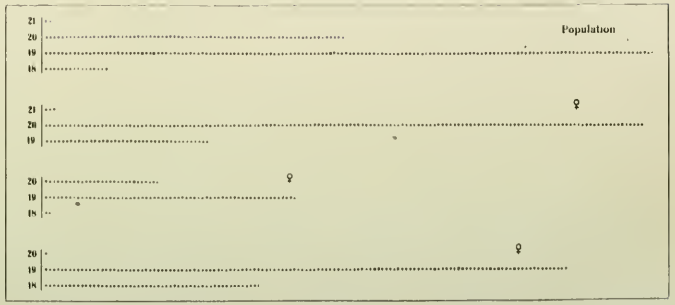
a = **89.41** Fl.= ± 0.51 | a = **93.37** Fl.= ± 0.57 | a = **88.08** Fl.= ± 0.36 | a = **85.97** Fl.= ± 0.43

Fig. 29. Dorsal rays (D).



a = **112.20** Fl.= ± 1.27 | a = **116.73** Fl.= ± 0.63 | a = **111.41** Fl.= ± 0.38 | a = **107.99** Fl.= ± 0.53

Fig. 30. Rays in right pectoral (Pd).



a (Pop.) = **19.26** Fl.(do.) = ± 0.13 | a_1 = **19.81** $Fl._1$ = ± 0.10 | a_2 = **19.29** $Fl._2$ = ± 0.18 | a_3 = **18.72** $Fl._3$ = ± 0.11

(A) and the total number of dorsal fin rays (D). The average values for these two characters in the populations from St. 31 in the Ise Fjord were 89.4 and 112.2 respectively. The population analyses are shown in the graphs Fig. 28—29, together with the results of some offspring analyses for the same character. As in the case of the vertebræ, so also here, the most common variate in the population is marked in the offspring graphs by a horizontal line, and the figures for the mother are noted in each offspring graph.

From these few examples — we have many more analyses to draw from — it will be seen that the average number of rays in the offspring samples may differ widely from the figures for the population. In the case of the anal fin, the average figures vary from 93.37 to 85.97 (the population average being abt. 89.4) and in the case of the dorsal fin, from 116.73 to 107.99 (population abt. 112.2).

As regards the pectoral fins (Pd and Ps), we find a similar state of things in the offspring analyses. This will be seen from the four graphs here given (Fig. 30) showing a population analysis from St. 31 in the Ise Fjord and three individual analyses of offspring from the same place. Only the right pectoral (Pd) has been included, but the left (Ps) shows the same results as the right.

We have here, it is true, the same variates, to wit 18—21, but

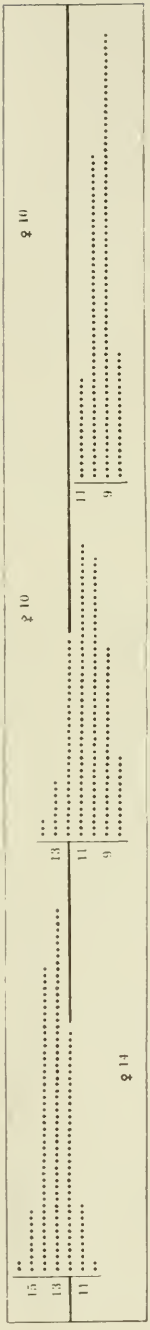


Fig. 31. — Stat. 17, Mariager Fjord. — Pigment spots ($Pigm D_1$). Individual offspring samples.

the average values may also vary very considerably for this character. Thus the figure for the population was 19.26, whereas in the individual offspring samples, it fluctuated between 19.81 and 18.72.

Finally, we have the pigment (PigmD_1). The few examples here given are not from the Ise Fjord, but from St. 17 in Mariager Fjord, where the population average is very low, abt. 11.8. It will be seen from the graphs (Fig. 31) that the three offspring samples may differ considerably from the population, and that their averages vary from 13.05 to 9.41

As regards the hard rays in dorsal fin (D_2), we have not yet made counts of large offspring samples, but there is no reason to believe that this character will turn out otherwise than the others with which we are concerned.

Altogether, we can assert that the individual offspring analyses may, and often do, give average values differing widely from the average of the population.

As we have seen in the previous section, the species *Zoarces viviparus* was found, by our population analyses, to be divisible into numerous local races.

The investigations described in the present section have carried us a step farther. They have shown, that the smallest unit hitherto considered, the race or population, can by individual offspring analyses be further resolved into still smaller elements. These, the individual offspring samples, can differ widely one from another, and each one of them may again present an average widely different from that of the population, but when taken together as a whole, they present the same picture as the population.

The state of things thus revealed by our individual offspring analyses is in several respects similar to that described by W. Johannsen, in his classical work "Über Erblichkeit in Populationen und in reine Linien" (1903) based on experiments with beans and barley, as also in subsequent publications. From the mixture composing an ordinary bean population, Johannsen was able, by offspring investigations on the principle of the "pure line", to isolate elements which were constantly different in respect of several characters, such as the weight and shape of the seed.

It is probably something of the same sort which takes place in our individual offspring analyses, whereby a given population of *Zoarces* is resolved into smaller elements. One point of difference, however, should be noted: the *Zoarces* are neither selfing, as beans and barley, nor can they, like these, be "cultivated" without difficulty throughout several generations. Consequently, it is impossible to carry our investigations to the degree of completeness and clearness attained by Johannsen by means of his "pure lines". This need not, however, prevent us from supporting our judgement of the problems we encounter on the results obtained in investigations with the selfing, homozygous plants.

VII. Some general results of the investigations on races of fish. — Survey and discussion.

A. The principal works.

We have in the foregoing set forth a series of new facts regarding *Zoarces*. Before proceeding to discuss the importance of these, it will be necessary to mention briefly the most important investigations hitherto carried out with other species of fish. There is no need for a lengthy description, as an excellent survey is already given in Redeke's two works (1912 and 1915).

Local races have in course of time been shown to exist in quite a number of fish species. Most important of all, in my opinion, are Heincke's herring investigations, collected in the comprehensive "Naturgeschichte des Herings", 1898. From a mere chaos, Heincke succeeded, by his admirable and systematic work, in furnishing not only a basis for all future investigations in this field; he also succeeded, through the study of a single species, the herring, in revealing so many important features, — quite unexpected in part — as to the occurrence and relationship of various races, that subsequent investigations with other species have in a certain degree only amounted to a repetition of Heincke's results. Of great theoretical interest, apart from the particular subject concerned, is Heincke's "Metode der kombinierten Merkmale", described in the same work, according to which it is possible to determine the race to which a single herring belongs, and this with a higher degree of certainty, the

greater number of characters taken into consideration, and the less their degree of correlation (cf. Duncker, 1908, p. 7).

After Heincke, racial investigations with herring have been carried out with great success by several investigators, among whom we may mention A. Cligny, J. Hjort, A. C. Johansen, E. Lea and H. Ch. Williamson. Other fish of the herring family also (*Clupea sprattus*, *Engraulis encrasicholus*, etc.) have been investigated in this respect, by Heincke, Hoek, Fage, Redeke, etc.

Particular attention has been devoted to racial investigations in the case of the plaice (*Pleuronectes platessa*) and the flounder (*Pl. flesus*). A series of British, Danish, Dutch and German scientists have here contributed important works, especially Cunningham, Duncker, Heincke, A. C. Johansen, Kyle, C. G. J. Petersen, and Redeke.

Distinct racial differences have, however, also been demonstrated in many other species beside the plaice and flounder etc. We may here mention *Gasterosteus aculeatus* (by Heincke), *Siphonostoma typhle* (by Duncker), *Anguilla* (by Johs. Schmidt), *Mullus barbatus* (by L. Fage), and many others. Further reference may be made to the introduction to Redeke's paper (1912).

B. The characters employed.

Common to all these works is the fact that the distinction of races is based upon characters which can be quantitatively measured. The races are therefore described as quantitatively different, and are characterised by the average value for the qualities concerned, which may be directly compared, since they are expressed in figures of the same denomination. Otherwise, the racial characters may differ very considerably.

A first group is formed by such characters as are expressed in whole numbers ("integrated variates"). To this belong for instance, the number of vertebræ, of fin rays, pigment spots, spines, scales, pyloric appendages, etc.

A second group is that of the characters which are not expressible by whole figures. Such are for instance the qualities indicative of the shape of the body, or of any part thereof, the position or size of an organ, etc. etc. As the measurements of these characters may give all possible values between any two whole

figures, they must be artificially grouped in classes ("graduated variates").

Finally, as a third group, we have the so-called physiological characters, among which are reckoned rate of growth, time of spawning, of reaching maturity, fertility, etc.

Obviously, it will be preferable to work with the first-named group of characters, partly because they are measurable with absolute accuracy, and partly because they are formed at a very early stage, and remain constant throughout the life of the individual, both of which features are of great importance. The reader will have observed that I have in the present work employed only characters of this group; I do not, however, in the least intend to imply that characteristic differences between the various *Zoarces* populations could not be discerned by means of other qualities

C. What do fishery-biologists understand by a "race"?

Up to the present, the word "race" has been used in this work without reserve to denote populations of *Zoarces* which could be characterised as distinct from others by their occurrence and average structural conditions. Are we now justified in employing the word in this sense; i. e. as a term for the smallest systematic unit found in our analyses of the *Zoarces* populations?

Heincke (1898, I, XCL), defines his systematic group of the first order, which he indiscriminately terms "Tribe", "Family", "Local Form" and "Race", all in contradistinction to "Species", as follows:

> "Familie oder Stamm ist eine Gruppe von Individuen, die an demselben Orte unter gleichen Bedingungen in gleichen Gewohnheiten leben und durch unmittelbare Kreuzung und Zeugung in engster Blutverwandschaft stehen."[1])

Such a definition would well apply to our *Zoarces* races, but it tells us nothing, and is hardly intended to tell anything, as to the nature of a race in itself.

Heincke has on various other occasions in the course of his large work on the herring put forward statements which afford an insight into his views as to this, but I have not been

[1]) Redeke (1915, p. 4) adheres entirely to Heincke's definition.

able to find any clear expression of the same, such as might for instance be translated into the terminology of modern heredity studies.

Duncker (1913, p. 231) draws the limits of the term race more sharply than Heincke, and distinguishes between "race" and "local form" as follows:

> "Unter einer Lokalform verstehe ich eine Gemeinschaft von Individuen derselben Spezies, welche sich an demselben Fundort aufhalten und von gleichzeitig lebenden Individuengemeinschaften dieser Spezies anderer (näherer oder entfernerer) Fundorte dauernd morphologisch unterscheiden. Unter eine Rasse verstehe ich eine Gemeinschaft von Individuen derselben Spezies, welche untereinander blutverwandt sind und sich, unabhängig von ihrem Aufenhaltsort, durch mehrere Generationen hindurch von anderen in sich blutverwandten Stämmen dieser Spezies in gleichem Sinne morphologisch unterscheiden. Beiden Begriffen gemeinsam ist also die morphologische Verschiedenheit der verglichenen Individuengruppen; ihr Unterschied liegt in der lokalen, im Gegensatz zur erblichen, Bedingtheit dieser Verschiedenheiten."

This definition is clearer than Heincke's, as it separates the hereditary element from that of the environment; unfortunately, however, our knowledge is here so inadequate, that we are not in a single concrete instance able to determine whether we have to deal with a "race" in Duncker's sense, or a local form. This applies as well to *Zoarces* as to all the other fish species in which racial differences have been found[1]).

[1] Duncker states, in the same work (1913, p. 230) that "ein Rassengegensatz ist unverkennbar zwischen der Ostsee- und der Nordsee-Scholle" — i. e. between the plaice of the Baltic and those of the North Sea. I have no doubt that he is right, though the only conclusive proof, to wit, experiment, is still lacking.

Heincke asserts (1916, p. 187) that the two German transplantation experiments with marked plaice (from the North Sea to the Baltic and *vice versa*) have proved, that "die verschiedenen Grade in der Schnelligkeit des Wachstums bei den einzelnen Lokalformen der Scholle wirklich konstitutionelle, erbliche Rassenmerkmale sind". This application of the term "erblich" (hereditary) however, appears to me not altogether justified. It is possible, and indeed probable, that we have here to deal with a hereditary quality, but the point is not proved until it has been shown that the offspring of the transplanted plaice produced under the new environment had the same rate of growth as the parent population By way of further illustration, I

The fishery biologists, then, use the term "race" in varying senses. In the present work, where I have spoken of races of *Zoarces,* it has not been my intention to assert that the average differences found by population analyses are throughout indicative of any hereditarily constant peculiarity. This, as a matter of fact, we do not know, either in the case of *Zoarces* or for the other species. It is possible that such may be the case, but the reverse is likewise possible, and a decision can only be arrived at by means of experimental investigations, which have not up to the present been carried out to a sufficient extent.

Under these circumstances I use the word race where others might perhaps write "local form" or another term, well knowing that there may be question as to whether such use is justified, but reserving a classification of the related terms for future discussion.

D. Generally accepted views as to the importance of environment.

We have seen that in the case of many species of fish it has been shown that populations of one and the same species living in different seas exhibit characteristic differences in structure and manner of life.

The principal cause of this difference is, as far as I can see, considered by most fishery biologists now working, as due to the environment, or in other words, to the varying external conditions under which the different populations have to live. We may easily find numerous expressions of this view in the literature; Heincke (1898, I, p. LXXI—LXXII) expresses it as follows:

"Alle meine Beobachtungen weisen übereinstimmend darauf hin dass ein enger Zusammenhang bestehen muss zwischen den charakteristischen Eigenschaften einer Heringsrasse und den besondern physischen und biologischen Verhältnissen ihres

may perhaps mention my cultivation experiments with an American hop plant, which differs from all European sorts by its peculiar aroma. This individual produces year after year hops whose aroma at once shows that they belong to a plant of American origin. But that the quality of "American aroma" is hereditary, and produced even in the Danish climate I was only able to prove by cultivation of the offspring produced in this country, from the plant in question. (Johs. Schmidt, "Investigations on Hops (*Humulus lupulus* L.)", No. V and No. X, Comptes-Rendus des Travaux du Laboratoire de Carlsberg, vol. 11, 1915 and 1917).

Wohngebietes. Wäre dem nicht so, dann könnten so zahlreich lokal begrenzte Rassen überhaupt nicht vorhanden sein. Die Thatsache, dass die eine Lokalform etwa im Mittel 57, die andere nur 55 Wirbel besitzt, kann keine zufällige sein, die im nächsten Jahre vielleicht ins Gegenteil umschlägt. Sie muss tief begründet sein in den verschiedenen Verhältnissen bei der Wohngebiete, und diese müssen sich wenigstens eine geraume Zeit hindurch stabil erhalten."

Writers are thus agreed as to the fact that the environment is of great importance, but there still remains the question as to how, in what manner, the environment produced such differentiation.

Generally speaking, the effect of environment may be imagined as taking place in two essentially different ways: either directly or indirectly. In the former case, we should have a direct action upon the separate individuals, or sexual cells as the case may be, taking place during the development of the same. If the environment be constant, then the result will be that the population year after year remains constant. In the other case, the action of the environment is of quite a different character. Without acting upon the separate individuals in the population, it makes itself felt by indirect means, inasmuch as it encourages certain individuals having certain definite qualities or combinations of such, in preference to others with other qualities. The result will then be that a selection takes place, whereby the average character of the population is changed according to the change of environment (cf. for instance Duncker 1908, p. 6—9).

This is, however, merely a schematic exposition, a point which should not be forgotten. In concrete cases, it will as a rule be impossible to determine, at any rate, without experimental aid, whether we have to deal with the one or the other form of influence; the direct, or the indirect (selective) action of environment upon population.

It is but rarely that any attempt has been made to explain differences between two populations as due to a selective process derived from the local environment. An instance of this is found in Duncker's last work on the plaice (1913, p. 234—235). He has here endeavoured to explain certain average differences between

the plaice populations in different parts of the North Sea by arguing as follows: When we have shown that such differences exist in respect of number of vertebræ and fin rays among the plaice populations at the different localities, then the difference cannot be due to a direct effect of the environment at the places where the fish grow up, since the full number of vertebræ etc. is already present in the larvæ, when they arrive from the breeding grounds at the scene of their future growth. It must therefore be supposed that a selection takes place after arrival there, so that individuals having certain definite numerical values or combinations of such for the characters in question are better able to live under certain external conditions than others. The result of this must then be, that certain variates would be exterminated at one place, others at another, whereby the average of the population would be altered[1]).

The differentiating importance of the environment is thus generally acknowledged by fishery biologists, but what external factors are the active ones we do not know. Among those which may be presumed to be of importance are generally reckoned the following: Salinity, Temperature, Depth, Nature of bottom, Nature and Quantity of food, etc. But as soon as we attempt to go even slightly into details in any particular case, we at once find that it is difficult to make the "explanation" fit in. This point has already been discussed at length by Heincke in his great work on the Herring (1898, p. LXXI *et seq.*) and I will therefore be as brief as possible here.

We may take, by way of example, the salinity, and consider this somewhat more closely in relation to races of fish. As is generally known, the salinity is very low (less than 10 ‰) in the inner parts of the Baltic, but rises as we move westward through the Danish waters to the North Sea (and the Norwegian Sea) and further to the Atlantic, where we find a salinity of over

[1]) By way of comparison, I may call to mind my investigations with the common eel (*Anguilla vulgaris*) (1913 and 1915). From these it appeared that all the eels of Europe were derived from breeding grounds in the Atlantic, and that the larvæ (or the elvers) long before reaching the coasts, had already their full equipment of vertebræ, fin rays, etc. Thus far, then the situation is comparable with what Duncker presumes for the plaice, but in the case of the eel there is nothing to indicate that any selection takes place, since all populations from Europe exhibit complete uniformity (cf. Fig. 20—21, p. 314).

35 ‰. Moving then from the Atlantic into the Mediterranean, the salinity further increases, up to over 39 ‰ in the eastern parts of that sea. In fjords and bays, the general rule is, that the salinity is greatest near the mouth, decreasing inwards towards the base.

Let us now endeavour to form some idea as to whether there can be any definite relation between the salinity and the average value of any well investigated character. By way of example, we can take the two best studied species, the herring and the plaice. In the accompanying table, reproduced from Heincke (1898 and 1916), are shown the approximate averages for number of vertebræ in plaice and spring herring from various waters, arranged in order of increasing salinity.

Average no. of vertebræ, after Heincke.

Water	Plaice (*Pleur. platessa*)	Spring herring (*Clupea harengus*)
Baltic	42.5	55.2
Southern North Sea . .	43.0	55.3
North-western North Sea	43.0	56.3
Iceland	> 43.0	> 57.0

It will be noticed that the table agrees well with the presumption that the number of vertebræ should be determined by the salinity, the figures for both species being lowest in the Baltic, and highest in Iceland waters. If then we had only investigated this one character, the number of vertebræ, and had we no other population analyses than these few here represented, it would seem justifiable to assume a causal relation between the salinity and the average number of vertebræ in the two species[1]).

Unfortunately, however, the matter is more complicated than this; in the present instance, as in so many others, the greater

[1]) A third species, the sprat (*Clupea sprattus*) exhibits according to Heincke (1898), the reverse, the number of vertebræ being greatest in the Baltic, lower in the North Sea, where the salinity is much higher. This need not, however, affect such a view in principle, as there would of course be no reason why the number of vertebræ in a species might not be inversely proportional to the salinity.

the number of observations, the more difficult it is to make them agree!

For the sake of brevity, I will here disregard the complications arising from the fact that several characters have been considered, and merely deal with the question of vertebræ in relation to salinity. Now as a matter of fact, the investigations actually made embrace, not only populations of herring and plaice from the waters mentioned in the table, but also from for instance the White Sea. Here, the salinity is abt. 25 ‰, i. e. greater than in the Baltic, but less than in the North Sea and the Atlantic. We have then to consider what the average number of vertebræ amounts to for populations from the White Sea.

In the case of the plaice, Heincke states (1916, p. 187) 43.5. If now the number of vertebræ stood in direct proportion to the salinity, we should not expect to find so high a figure in the White Sea as at Iceland, where the salinity is higher; such is, however, the case. We have here, then, already a breach of the rule, the number of vertebræ in the plaice from the White Sea being too high in proportion to the salinity.

For the herring from the White Sea, the average number of vertebræ is according to Heincke (1898, p. XXIII) 53.6, i. e. far lower not only than in Iceland waters, but also than in the Baltic, where the salinity is so low. Here then, we have a new exception to the rule, but in the opposite direction, the number of vertebræ for the White Sea population being this time too low in proportion to the salinity.

We have not anything like the same number of population analyses from different regions for other species as for the herring and the plaice, but we shall see later on that similar breachs of the "rule" occur in the case of *Zoarces*. Among other investigations, Duncker's fine work on the pipe fish *Syngnathus typhle* (1908) deserves mention here on account of the very precise results attained. Duncker investigated three populations; from the Baltic (Neustadt Bay), from the Channel (Plymouth) and from the Mediterranean (Naples). In the first named locality, the salinity is below 20 ‰, in the second 35—35.5 ‰, and in the third abt. 37—38 ‰. The number of vertebræ (total sum of the rings) was, for the localities in the order above given, abt. 52.8, abt. 55.5, and abt. 54 8. Had the salinity been the determining factor, we should have expected to find the highest figure either in the

Baltic or in the Mediterranean, while as a matter of fact it occurs in the English Channel, i. e. at an intermediate degree of salinity. Had a greater number of populations been examined, it is likely that still further exceptions from the rule would have been discovered.

For the average number of pectoral rays, Duncker found the following figures: abt. 13.4 (Baltic), abt. 14 8 (Plymouth), and abt. 16.0 (Naples). Here, then the figures lie in the "right" order as regards salinity, but with the rays in the dorsal fin it is another matter, the figures here being abt. 34.9 (Baltic), abt. 37.8 (Plymouth) and abt. 34.4 (Naples). For more convenient reference, the figures are shown together in the accompanying table.

Syngnathus typhle. — Average no. of vertebræ (Vert), dorsal rays (D) and rays in right pectoral (Pd), after Duncker (1908).

Water	Vert (Total no. of Rings)	D	Pd
Baltic	52.8	34.9	13.4
Plymouth	55.5	37.8	14.8
Naples	54.8	34.4	16.0

These few examples will serve to show how hopeless it is to seek for any fixed rules as to the relation between average racial characters and salinity. True, we can at times discern something approaching a rule, but the more populations we investigate, the more exceptions we find, and this will presumably be the case to a higher degree as the species dealt with is more particularly local.

After this result of our endeavours to connect the average racial characters with the salinity, we may doubtless save ourselves the trouble of examining the other external factors in like wise. For the salinity is after all the one we know most about of those concerned. Otherwise, it might be natural to glance at the temperature, which is possibly also of much more importance than the salinity, but this, as we know, fluctuates considerably during the course of the year, and I am unable to give any detailed survey of the temperature conditions for the different waters. As regards the other factors which might be considered,

we have not sufficient knowledge to go into the question further in this connection.

Heincke, in his work on the herring (1898) has, as mentioned above, discussed at length the question as to influence of environment, especially salinity, upon the average racial characters. He calls attention (p. LXXII—LXXIII) to the apparent connection between the gradual alteration in the average value of various characters in the herring and the decrease in salinity from the Baltic towards the North Sea, but points out emphatically at the same time, that there are exceptions to the rule. Among the examples thus cited we find for instance the herring of the White Sea, which have a lower number of vertebræ, etc. than would be expected from the salinity of the water. Heincke attempts to explain the discrepancy by supposing that the effect is not produced by the one factor, salinity, alone, but by the manner in which this and other external factors are combined. He writes:

> "Ein niedriger Salzgehalt des Brutwassers verbunden mit hoher Wärme wird ein ganz anderes Ergebnis liefern als ein niedriger Salzgehalt verbunden mit geringer Wärme."

I will not go further into this question here, as it will be taken up again in the following section, but would merely say that in my opinion there is nothing gained by such a supposition. It does not simplify matters, and is in reality only another way of stating the simple truth, that we really understand nothing at all about these features.

It should be noted, however, that both Heincke and others who have studied questions of this nature at length, emphasise more or less the necessity of experiment as the only possible means of coming to a decision. On this head, there seems to be a general unanimity among investigators from all sides.

VIII. What general conclusions may be drawn from our *Zoarces* investigations?

We may now finally consider whether the facts revealed by these *Zoarces* investigations lead us to any conclusions of a more general character. First of all we may put the question: Is it possible to find any relation between the average charac-

ters of the different races and their environment? Here again, the lack of other data leaves us only the salinity to consider.

Among the populations analysed, that from St. 57 in the North Sea (Scotland) is one of those living in water of highest salinity, that from St. 52 in the Baltic (Hudiksvall) the one whose surrounding salinity is lowest. The difference between the two stations is very great, the salinity off the coast of Scotland being abt. 34 ‰, and at Hudiksvall only abt. 5 ‰. Thus the population at St. 57 lives in a salt solution more than six times as strong as that at St 52.

The graphs in Fig. 32 show the number of vertebræ for these two populations. It is at once noticeable that there is no difference worth mentioning between the two, either as regards course or average value, the latter being 116.1 and 116.4 respectively. The number of pectoral rays and number of pigment spots are also about the same for the two populations, but the number of hard rays, on the other hand, differs considerably.

Another example is furnished by the two graphs in Fig. 33. The figure here shows the number of vertebræ for two populations living in water of about the same salinity, a comparatively high value in both cases (abt. 30 ‰). It will immediately be seen that despite the uniformity in respect of salinity, there is a very marked difference between the two populations, the one (St. 1, Læsø) having an average value of abt. 117.3 for vertebræ, the other (St. 8, Limfjord) only abt. 111.2.

Even more striking perhaps is the third case, illustrated in Fig. 34. The two populations whose vertebræ averages are here shown are from Roskilde Fjord, Sealand and Kjelds Nor, Langeland, respectively, i. e. from water of uniform, comparatively low salinity (abt. 12 ‰). And it is at once apparent that there is an enormous difference between the two populations, amounting to no less than 11.5 vertebræ in the average.

The graphs further show that the two population analyses did not reveal a single common variate. The range of variation for Roskilde Fjord, for instance, was 103—113, that for Kjelds Nor 114—124. Here then we have a dissimilarity so great that those unacquainted with the facts might well believe two distinct species were concerned, and this between two populations living within the same area of sea, and as far as we know, under uniform external conditions.

Fig. 32. — Stat. 52, Gulf of Bothnia, and Stat. 57, Scotland. — Vertebræ (Vert). Shows the number of vertebræ of two populations living at very different salinity, respectively about 5 ‰ (Stat. 52 and about 34 ‰ (Stat. 57).

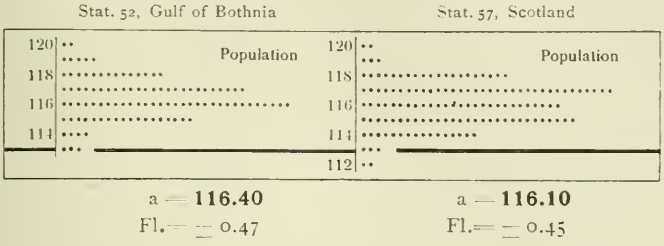


Fig. 33. — Stat. 1, Læsø, and Stat. 8, Limfjord. — Vertebræ (Vert). Shows the number of vertebræ of two populations living at a rather high salinity of equal amount (about 30 ‰).

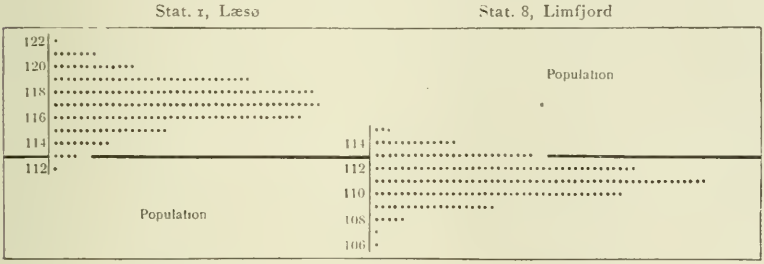
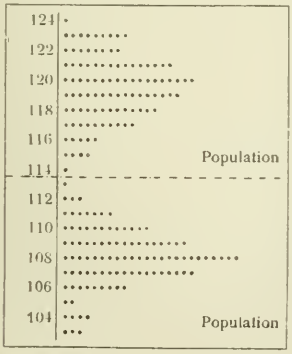


Fig. 34. — Stat. without no., Kjelds Nor (Langeland[1])) and Stat. 34, Roskilde Fjord 1916. Vertebræ (Vert). Shows the number of vertebræ of two populations living at a low salinity of equal amount 12 ‰). — The two populations are separated by a dotted line.



Kjelds Nor 1916
a = **119.44**: Fl. = ± 0.75

Roskilde Fjord 1916
a = **108.06**: Fl. = ± 0.69.

[1]) Station Kjelds Nor is not marked in the charts and lists in Section X. It is situated a little south of St. 43 (see Chart, Pl. II).

These few examples might be supplemented with many others, but it is not necessary to pursue the subject further. What we have already seen is sufficient to show that we may in water of like salinity find populations with highly dissimilar characters, while on the other hand, like characters may occur in water of widely different salinity.

Our investigations thus offer no support for the theory that racial character averages should be primarily determined by the environment. In particular, they show that the salinity is hardly to be regarded as having the importance generally attributed thereto. And though the point is perhaps more clearly evident from the investigations with *Zoarces* than from previous researches, this is certainly not due to the existence of any difference in principle between *Zoarces* and the other species Apart from the presence of an extensive material for analyses, the reason must be sought in the fact that our present species is more local, and exhibits greater average differences between one population and another, than is the case with those previously investigated.

Is there, then, nothing in the results of our *Zoarces* experiments which might indicate that the environment does count for something in determining the average values for the characters we have considered?

Save for experiments, we have, as far as I can see, no other means of deciding the question than by endeavouring to ascertain how far any gradation of characters can be found to exist, in connection with a gradation of the environment. To a certain extent, this has already been attempted. We have seen that it was not possible to find any such, at any rate, not while considering the entire large area within which *Zoarces* occurs, as a whole. The question then is, whether a different state of things may be brought to light by considering only populations living near one another, within a restricted area. Is it possible, within such reduced areas, to find any relation between the environment and the average characters of the populations? This question must be answered in the affirmative, as will be seen from the investigations of the fjord populations mentioned in Section V, D, p. 301—310.

We found, it will be remembered, that the average number of vertebræ was always lower up in the fords than

in the waters outside. The graphs in Figs. 6, 10, 11, 12 and 15 at once make this apparent, and the same will also be seen from the chart Pl. II.

In this distribution or gradation it is hard to find anything but an expression of the importance of environment. True, the finding of a lower average value up in one or another fjord need not absolutely imply that it is the fjord environment which has brought the figure down. We might perhaps in another fjord find that the reverse was the case, i. e. a higher value up in the fjord than outside. As a matter of fact, however, the populations up in the fjords have everywhere a lower average number of vertebræ than those outside. This statement must, of course, be subject to the reservation: as far as our investigations go up to now, but we have already a considerable number of examples, and all tend in the same direction. There can thus hardly be any question of "chance", and we are consequently constrained to conclude, from these fjord investigations, that the water up in the fjords has, or lacks, certain "factors", differing therein from the waters outside, with the result that *Zoarces* in the former case have fewer vertebræ than in the latter. Which these factors are, however, or in what manner they affect the population, — whether directly by acting on each separate individual, or indirectly by selection, — we are at present unable to say, except as mere conjecture. Experimental investigations will be required if we are to push our knowledge further.

Our fjord investigations then, lead us to the conclusion that environment may, at any rate to some degree, exert a determinative effect upon the average values of the characters investigated. And thus far we are in agreement with Heincke. We cannot, however, without reserve, follow him and other fishery biologists so far as to regard the environment as the only, or the essential, source of difference in the qualities of races[1].

[1] I must, however, repeat what I have already said (p. 327—28) that Heincke's views as to the causes of racial difference are not altogether clearly expressed. That he regards the environment as of decisive importance seems beyond doubt; this is evident from the passage quoted on p. 329 and also from many other statements in his large work on the herring. We may for instance quote the following (1898, p. LXXIX): "Wenn die Rassen das Ergebnis bestimmter Lebensbedingungen sind ....", and: "nach dem ganzen Bilde zu urteilen, was die gegenwärtigen Rassen mir gewähren, glaube ich, würde diese Umvandlung der alten Formen in neue durch direkte Wirkung der veränder-

As mentioned on p. 336, we found the same average values for several important characters in two populations from widely differing environments, *viz.* St. 57 (north-western North Sea) and St. 52 (inner Baltic waters). Other population analyses again, produced results such as those shown in Fig. 33—34. And from such data as these, I can draw no other conclusion than that other factors besides the environment must have contributed to the production of the averages in question.

Heincke will, I presume, explain such "irregularities" and "exceptions from the rule" as these and many others pointed out by himself (e. g. the remarkable figures for the herring of the White Sea) by supposing that the values found are due to various combinations of external factors. Such a theory, however, easily leads us beyond control, and seems therefore less promising for our purpose. And moreover, I hardly consider it necessary. It would seem more reasonable to suppose that the racial differences are, — to adopt the terms used by W. Johannsen (1913 for instance) — in certain cases of a genotypical nature, in others phænotypical only.

The correctness of this supposition may to a certain extent be experimentally tested, but the experiments required have not yet been brought to a conclusion, and until their results are available, nothing can be stated with certainty. The following remarks, therefore, are only to be regarded as an attempt to lead the discussion as to racial questions among fishes and other organisms in a new and, it is to be hoped, a productive direction.

Let us suppose the *Zoarces* to be normally selfing, and thus homozygous. A *Zoarces* population would then consist of individuals belonging to a large number of different "pure lines",

ten Lebensbedingungen herbeigeführt werden." Further (1898, p. CIV): "Nur dieser Annahme von dem direkt umbildenden Einflüsse der äusseren Lebensbedingungen fügen sich alle thatsächlichen Beobachtungen ohne Zwang." In a recent work, a survey of the races of plaice, 1916, Heincke expresses himself in a manner less easy to comprehend. We find here (1916: p. 186), the following: "Solche deutlichen und charakteristischen Wachstumsunterschiede können unmöglich rein vergängliche und zufällige sein, sie müssen vielmehr in den physischen und biologischen Eigentümlichkeiten der einzeln Meere begründet und somit dauernde sein und auf vererbter Konstitution und vererbten Lebensgewohnheiten beruhen." In cases such as this one cannot but feel strongly the lack of that precise terminology which is employed in modern genetic studies.

each with its own combination of genes. One line might for instance be genotypically equipped with 122 vertebræ, 16 pigment spots and 8 hard rays, a second might have say 120 vertebræ, 12 pigment spots and 12 hard rays, while a third appeared with 108, 14 and 6, and so on; in a word, all possible combinations. Within each pure line, the given combination would, by continued self-fertilisation, be maintained constant from generation to generation, but if alien fertilisation should ever take place, then other lines with new combinations would arise.

On analysing the offspring of a selfing, homozygous *Zoarces* in the same manner as we have employed in our individual offspring analyses, we should probably obtain a result similar to those here shown (Figs. 24—31), with the variates arranged according to the curve of coincidence, but possibly with a somewhat reduced field of variation. All offspring individuals, however. would, despite their difference one from another, have the same genetic formula, to wit, that of the mother, and the differences would thus be only phænotypical. The variation found would then necessarily be due to the more or less abundant nourishment of the different offspring individuals in the ovary, or other "external" factors, and would therefore have to be reckoned under the heading of "fluctuating" or "individual" variation. Specimens of the next, and of all succeeding generations would, despite considerable individual differences, still have the same genetic formula, as long as selfing took place.

What sort of picture might we then expect to obtain from analyses of *Zoarces* populations, provided that the conditions were as here supposed? I believe it would be exactly the same as that shown by our population analyses. The average values there found for the separate characters (the average "racial characters") would differ for the different populations, and would depend upon the mixing or quantitative proportion between the genotypes represented in the population. The "race" would to a great extent be merely a statistical term, indicating a mixture of genotypes in a proportion which, though differing as between one race and another, would nevertheless be constant within each from generation to generation, even disregarding the environment of the year-class and locality. If the environment of a race varied from one year to another, then the different year-classes might naturally exhibit phænotypical differences in the character averages,

but this would not affect the mixing proportion of genotypes characteristic of the "race".

And now with regard to the individual offspring analyses. What result could we expect to obtain from these, if the conditions were as supposed? It would, I believe, be similar to what we have actually found. The results of the individual offspring analyses for three populations are shown in Section XII, p. 390—392. They suggest that populations can, as found by W. Johannsen in the case of his bean populations (1903) be resolved into smaller elements, here expressed by the averages of the decades. Despite the fact that the average number of vertebræ in the three populations differed very considerably, the values being abt. 117.4, 113.4 and 109.5 respectively, the same smaller elements may be found in all three populations, but the quantitative proportion naturally varies greatly, and decisively as to the average for the population, i. e. the "racial character".

As a matter of fact, however, *Zoarces* populations are not really mixtures of individuals belonging to a number of "pure lines". They are undoubtedly communities of hybrids, each having its peculiar genotypical formula, and being heterozygous for a large number of genes.

Nevertheless, this need not prevent our applying the point of view above marked out. The main point here was, that the populations are mixtures of a series of different genotypes in varying proportion, but constant for each separate race. And here it is only a question of the numerical proportion between the different genotypes; in principle, it is of no importance whether the genes in question are "borne" by homozygous selfers or by heterozygous hybrids.

The starting point of the view thus sketched out was the result of our individual offspring analyses, together with the results obtained by W. Johannsen in his work on selfing plants (1903). As already indicated, our analysis could not be carried out with anything like the degree of completeness attained in Johannsen's work, where the offspring of a single individual are compared throughout many generations. Up to now, we have been forced to content ourselves with two generations, and cannot hope to get out the smallest systematic units, which Johannsen was soon able to isolate, and the continued cultivation of which enabled him to prove the permanence of the type. If I

am correct in taking the results of our individual offspring analyses as the first steps towards an isolation of the genotypes in the populations, then it seems to me quite justifiable to regard the bean populations and the *Zoarces* populations as collateral, and consider both as mixtures of a series of different genotypes.

Bearing in mind the fact that our individual offspring samples are derived from the union of two highly heterozygous individuals, it seems likely that the variation within the separate samples (see Fig. 23, p. 317 and 25—31, p. 318—323) is not due exclusively to the effect of external factors. We must suppose that it is quite as much a result of the different combinations of mendelizing genes, which must be produced where two parents are markedly heterozygous. We must for the present leave in abeyance the question as to how a genotype with for instance 120 vertebræ should be produced — possibly by the coincidence of a number of independently mendelizing genes, as so acutely demonstrated by the Swedish genetic expert H. Nilsson-Ehle in the case of the red grain colour in wheat — or by other means. We hope, by continued experiment, to go further into this question, so that we may be able for instance to construct the genetic formula of a parent pair in a given case, when we have investigated their qualities and those of the offspring produced.

Future experiments must also decide how far my interpretation of the results obtained by our individual offspring analyses is correct. If so, then there can hardly be any doubt that what we have found in the case of *Zoarces* is generally applicable to all populations of fish etc. If, for instance, it were possible to carry out individual offspring analyses within two different populations of plaice, we should then expect to find in the one population offspring samples where the average number of vertebræ, let us say, differed greatly from that for the population as a whole, and might even be identical with that for the other population, etc. etc.

I would here call to mind the passage quoted from Heincke on p. 330. According to his view as here expressed, the fact that one race of herring averages 57 vertebræ, the other 55, cannot be due to accident, but must be deeply rooted in the difference between the external conditions under which the two races live.

If now my view, that the average character of races is

primarily only the statistical expression of an unequal mixture of different genotypes, be at all in accordance with reality, then there is naturally nothing to prevent our regarding the fact of one herring race having 57 vertebræ, while the other has 55, as the result of accident. The more local a race is, the more "irregularities" in the geographical gradation of the qualities must be expected to appear as the result of accident, when the number of population analyses is increased.

We have in *Zoarces* seen several instances of such "breaks" or "irregularities" in the geographical distribution of the characters, which I should be disposed to regard as due, not to the effect of environment, but to mere accidental peculiarities in the mixing proportions of the genotypes.

In this connection we may also call to mind the frequently noticed fact that two races which are widely separated in geographical respects may be far more similar one to another than either is to races from localities more nearly adjacent. I have thus shown (1913), that the Japanese Eel is far nearer the European than the latter to the American, although the Japanese population is separated from the European by an enormous distance, while the larvæ of the latter form are so little removed geographically from the American, that we have actually, in the Atlantic, taken both in one and the same haul. We may here also mention Heincke's interesting demonstration (1898, p. LXV—LXVI) of the fact that the herring of the White Sea have their nearest relatives, not in the races from Norwegian waters or other coasts of Europe, but in a race found on the east coast of Japan. The close relationship between races so widely apart in geographical respects seems to me a new indication of the fact that racial differences can by no means be explained as always due to differences of environment, but must often be primarily of genotypic origin.

For all this, however, it is far from my intention to depreciate the value of environment as a determinative factor in question of form. This I do not in the least mean to imply.

I have, indeed, myself been able, by experiments which I hope on a later occasion to describe further, to prove with certainty in several cases that a change of environment was capable of bringing about alterations in the structural conditions not only of *Zoarces* but also of other species of fish. And, as already

mentioned, numerous observations of natural conditions seem to point in the same direction[1]). I merely wish to point out that we are hardly justified in regarding all the racial differences which have in course of time been shown to exist among fish species, as exclusively due to the effect of environment. Everything seems to suggest that they should be regarded as the result of co-operation between certain "internal" and "external" factors; in other words, that they are of both genotypical and phænotypical nature.

Finally, I would once more emphasise the necessity of experimental work in this field. If we are to arrive at a closer understanding of the nature of races in fish, there is no other way but that of biological experiment. The variational-statistic methods, which have of late years been so largely employed in studying the races of food fishes, have proved of the highest importance. This is, in my opinion, primarily due to the systematic arrangement or classification of the material brought about by the adoption of the method in question. Also, of course, to the fact that it enables us in special cases of particular fishery-biological interest to determine with certainty that we have to deal with two or more different populations, which must consequently be regarded as local, and each leading a separate existence. As to the causes of such differences, however, the variational-statistic method, which does not enquire into the nature of the material dealt with, affords but little information. It is here that biological analysis has its place, and we cannot be too cautious in guarding against the belief that it is possible even in part to replace it by mathematical methods.

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[1]) We may here also mention the *Atherina Riqueti* described by Roule (1902) from the "Canal du Midi" in the south of France. This species is regarded by Roule as a genetically constant form of recent origin, "faite par l'adaption aux eaux douces d'un être marin" (to wit, *Atherina Boyeri*). Further, the finding, at Laachersee, Eifel, mentioned by Thienemann (1911 and 1912) of a *Coregonus* form differing in the number of the gill-rakers from the form (*Coregonus fera* Jur.) found in the Bodensee, from which the Laachersee specimens are believed to be descended, etc. etc. I may return to the discussion of these and other similar cases on a subsequent occasion.

IX. Summary.

The present paper is concerned with *Zoarces viviparus* L., the common viviparous blenny, a species of very common occurrence in the Danish waters, where it is found in shallow places, for the most part inside the 10 metre limit. Its area of distribution lies distinctly in north-eastern Europe, as will be seen from the chart Pl. I, where the limits of its regular occurrence are marked. The species is met with from the White Sea in the North to the English Channel in the South, along the coasts of the European continent and the east coast of Great Britain. On the east, it penetrates into the innermost parts of the Baltic, but on the west, it is already found to be rare along the west coast of Great Britain, and is not known with certainty from Ireland. A remarkable feature is the fact that it is lacking at Iceland and the Færoes (Section II).

On the coast of North America, from Delaware to Labrador, another species of *Zoarces* is found, *viz*; *Zoarces anguillaris* (Peck.), which is said to attain a length of 1 m. and a weight of 5 kg., and is thus essentially larger than the European, of which we have not seen specimens over abt. 40 cm. Finally, there is a third species, *Zoarces elongatus* Kner. This has been found in the north-east of Asia, and appears to be more nearly related to our present species than is the American form (Section II).

Zoarces viviparus has not previously been subjected to racial investigations. That it was selected in the present case is due to the following considerations: 1) it is of highly local occurrence, 2) it exhibits great variation, 3) it is viviparous, and comparatively easy to work with experimentally, 4) it is a common article of trade in Denmark, so that material could be procured without difficulty (Section I).

The characters subjected to thorough investigation are the four following: Number of vertebræ (Vert), of rays in right pectoral fin (Pd), of hard rays in dorsal fin (D_2) and of pigment spots on the anterior, soft-rayed part of the dorsal fin ($PigmD_1$). In certain particular cases, several other characters were also investigated (Section III).

The four characters above mentioned vary greatly. Thus the number of vertebræ may fluctuate from 101 to 126, number

of pectoral fin rays from 16 to 22, of hard rays from 0 to 17, and of pigment spots from 7 to 22. The number of vertebræ exerts a marked effect upon the shape of the body, specimens with many vertebræ being slender and elongated in form, while those with few are short and clumsy in build. The number of hard rays and of pigment spots may also be of physiognomical importance. (See sketches Figs. 16, 17 and 18, p. 309, 312).

The investigations fall into two groups: 1) Population analyses and 2) Individual offspring analyses (Section V and VI).

In the population analyses, we endeavoured, by investigation of representative samples from the respective populations (communities) of *Zoarces*, to arrive at some knowledge of these, and procure a survey of the different races and their distribution. A material of some 15,000 specimens, from 61 different places (Stations) was analysed, and dealt with according to the variational statistic method. As far as possible, abt. 200 specimens from each station were analysed. The position of the different stations may be seen from the charts Pl. I—II and the lists in section X, p. 351—359. The results of the population analyses are shown in detail in the tables Section XI, p. 360 *et seq.*

In these will be found noted for each of the four characters and each of the stations investigated, the following features: number of the different variates, average (a), standard deviation (σ) and fluctuation of average (Fl.). For the character of pectoral rays (Pd), we have further noted the percentage of males (♂) in each sample.

The average values have also been marked on chart Pl. I and II, each character in a separate colour: vertebræ in red, pectoral rays in blue, hard rays in green, and pigment spots in black.

Finally, the figures in text present several examples of population analyses in graphical form. All the graphs illustrating population analyses are marked "population".

In the course of preliminary investigations it was found that there was no difference, or at any rate, none worth mentioning, between the two sexes as regards the characters with which we are here concerned (Sections IV and XI).

The population samples investigated contained individuals belonging to several year-classes, the three-year-old fish being

as a rule the most numerous, but those of two and four years also frequent.

By taking and analysing samples from the same population at different times during the course of three successive years, we endeavoured to ascertain the constancy of the racial picture. These repeated population analyses revealed a high degree of constancy, as will be seen from the graphs in Fig. 1—5, p. 296—297. By way of example it may be mentioned that the average figures for number of vertebræ in the population of St. 31 in the Ise Fjord during the years 1914, 1915 and 1916, were 113.42, 113.23 and 113.40. Such repeated population analyses should, however, be carried out for a still longer period of years before the question can be regarded as finally decided (Section V, B).

Analysis of the 61 population samples showed that the average characters may vary greatly from place to place. The number of vertebræ for instance was seen to vary in the average from 119.2 (St. 43) to 107.98 (St. 34) and similar results were found for the other characters, the variation being least in the case of the pectoral rays, where we very rarely find more than four variates in one and the same sample, while the entire material only comprises 7 (16—22).

The average values for the different characters may be very differently combined in the different populations, as will be seen from the charts Pl. I—II. Disregarding the pectoral rays, where the range of variation is but slight, and taking *A B C* as indicating a high, *a b c* a low figure for average number of vertebræ, hard rays and pigment spots, we find that six out of the eight possible combinations are represented in our 61 population analyses: *viz*; *A B C*, *A B c*, *A b C*, *A b c*, *a b C* and *a b c*. Up to now, we have met with no examples of the combinations *a B c* and *a B C*, and among the six which did appear, not all were found to occur with like frequency (Sect. V, E).

An attempt was made to characterise the different large areas of sea according to the average qualities of their respective populations, and it was found that this can, to a great extent, be done. On dividing the whole region into four parts, and using the same symbols as above, we obtain the following: 1) Western North Sea, *A b c*, 2) Eastern North Sea, *a b c*, 3) West Baltic area, *A B C*, 4) East Baltic area, *A B c*. A survey of the average values within each of these four areas is given in the chart Pl. III (Sect. V, C).

On going into details, we meet with many divergencies and irregularities in the geographical distribution of the average figures. Particularly remarkable are the conditions encountered in the shallow fjords. The rule here is that the average number of vertebræ, hard rays and pigment spots is lower in the fjords than outside. Various instances were quoted in illustration of this. (See chart Pl. II, and graphs Figs 6—15). The lower number of vertebræ renders the physiognomy of the fjord *Zoarces* very different from that of the coast populations, the former being of short and heavy build, while the latter are long and slender (cf. Figs. 16—17) (Sect. V, D).

On comparison with the eel, *Anguilla*, which has about the same number of vertebræ etc. as *Zoarces*, we find that there is great difference between the two species. *Zoarces viviparus* is divided up into numerous local races, whereas all the European eel populations are identical (see graphs Figs. 20 and 21). The explanation of this must be sought in the biological differences between the two: *Zoarces* spends all its life within the same narrowly restricted area, whereby intermingling of the populations is excluded. In contrast to this, all the ripening eels of Europe annually journey thousands of miles out into the Atlantic, where they meet and breed, the young subsequently returning to the European waters. Long before they reach our shores, they have already their full complement of vertebræ etc., and we have thus here a natural explanation of the fact that all the eel-populations of Europe are identical, i. e. belong to one and the same "Race" (Sect. V, F).

Zoarces viviparus, as its name implies, brings forth its young alive, and produces a considerable number, up to 400 per individual. Long before birth, which takes place in winter or spring, the offspring have developed their full quota of vertebræ, rays and pigment spots. Consequently, it is possible in the case of this species to make individual offspring analyses, each offspring sample being separately investigated.

Examples of such individual offspring analyses are shown graphically in Figs. 23 and 25—31, p. 317—323. The tables in Section XII moreover, p. 390—392, give the average number of vertebræ for each offspring sample from all the gravid females in samples from three selected populations (Stat. 31, 33 and 22).

The average number of vertebræ etc. in the offspring samples may vary greatly within one and the same population, and may

also differ greatly from the population average. By way of example, we have quoted three offspring samples from the population at St. 31 in the Ise Fjord. The average number of vertebræ for this population is abt 113.2—113.4, whereas the figures for the three offspring samples were abt. 117.8, 112.4 and 109.5 (see Fig. 25, p. 318).

My interpretation of the population analyses, as supported by the results obtained by W. Johannsen in 1903 with bean populations, is as follows. By means of population analyses, we were able to subdivide *Zoarces viviparus* into numerous local races. The individual offspring analyses showed that the smallest unit hitherto considered, the "Race", can be resolved into still smaller elements expressed by the averages of the offspring samples. These minor elements may differ widely one from another, and also from the average of the population (the "Race"), but when taken together as a whole, they present the picture of the race, as this is expressed by our population analyses (Section VI).

In Section VII mention is made of the most important researches concerning the question of races among fish, and the works of Heincke are here quoted as of fundamental importance both in respect of the methods employed and in the results attained. The question of ulterior causes of racial differences is discussed on the basis of the investigations made, and the discussion is continued in Section VIII, the results of our *Zoarces* investigations being taken into considcration.

In dealing with the problem as to importance of environment, several examples are quoted (see for instance the graphs Figs 32—34, p. 337), from which it would seem that difference of environment is not sufficient in itself to account entirely for the structural differences between the races, and that the importance of salinity in particular has been greatly over-estimated.

It is pointed out that the term "Races" of fish, as characterised by our population analyses, is only a statistical expression. The "Race" is a mixture of different genotypes, where the proportion between these primarily, the environment only secondarily exerts determinative effect upon the average values which characterise the race.

Finally, it is pointed out that we cannot expect to attain any deeper comprehension of the biological problems concerned by the employment of variational statistics alone. We can by

this means arrange and classify our material, but that is all, and it is only by biological experiment that we can hope to arrive at a knowledge of its nature.

X. List of the populations investigated.

St. 1. Læso, abt. $\begin{cases} 57^0\ 14{,}3' \text{ N.} \\ 11^0\ 10{,}4' \text{ E.} \end{cases}$

Aalebjergsdyb, depth 2 m. 22 June 1915.

From Fiskeriassistent N. Krogh, Frederikshavn.

St. 2. Hals, abt. $\begin{cases} 56^0\ 59{,}3' \text{ N.} \\ 10^0\ 20{,}5' \text{ E.} \end{cases}$

Abt. 1,5 km. N. of entrance to Limfjord in the so-called Indersø, inside the first reef. Depth nearly 2 m. Abt. 200 m. from Land. 5 Oct. 1915. Taken in eel traps by Th. Winther, Hals.

From Fiskerikontrollør L. Andersen, Aalborg.

St. 3. Limfjorden, east of Aalborg, abt. $\begin{cases} 57^0\ 04{,}7' \text{ N.} \\ 10^0\ 01{,}7' \text{ E.} \end{cases}$

East side of Bredhage, between the two eastern brooms. Depth 3 m. 16 Aug. 1915. Taken in eel traps.

From Fiskerikontrollør L. Andersen, Aalborg.

St. 4. Limfjorden, Nibe Bredning, abt. $\begin{cases} 57^0\ 01' \text{ N.} \\ 9^0\ 39{,}6' \text{ E.} \end{cases}$

Eastern part of the Bredning, nearly facing Binderup Aa. Depth nearly 4 m. 26 July 1915.

From Fiskerikontrollør L. Andersen, Aalborg.

St. 5. Limfjorden, Aggersund, abt. $\begin{cases} 57^0\ 00' \text{ N.} \\ 9^0\ 18' \text{ E.} \end{cases}$

Depth 1½—2 fathoms. 30 Oct. 1916. Taken by fisherman Jens Hansen, Logstør.

From Fiskerikontrollør L. Andersen, Aalborg.

St. 6. Limfjorden, Hjarbæk Fjord, abt. $\begin{cases} 56^0\ 33{,}5' \text{ N.} \\ 9^0\ 21{,}5' \text{ E.} \end{cases}$

Extreme eastern part of Hjarbæk Fjord between Lynderup and Skals. Depth 5—6 ft. 14 June 1915. Taken in eel hand-seine.

From Fiskeriassistent J. Jensen, Nykjobing.

St. 7. Limfjorden, East side of Mors, abt. { 56° 48,2' N. / 8° 53,5 E.

Taken in stake net N. of Nykjøbing. 6 May 1915.
From Fiskeriassistent J. Jensen, Nykjøbing.

St. 8. Limfjorden, Sallingsund, abt. { 56° 44' N. / 8° 50,6' E.

Between Langerhage and Glyngøre. Depth abt. 3 faths. 9 Nov. 1915. Taken in eel traps.
From Fiskeriassistent J. Jensen, Nykjøbing.

St. 9. Limfjorden, Nissum Bredning, abt. { 56° 43,3' N. / 8° 17,5' E.

Abt. 1 naut. mile W. of Kobberød. Depth 4—9 ft. 17 July 1915. Eelgrass with patches of sand.
From Styrmand H. C. Christensen, Lemvig.

St. 10. Nissum Fjord, abt. { 56° 24' N. / 8° 08' E.

Western part of fjord, abt. 2 miles north of the Thorsminde outlet. Depth 2 ft. 7—8 Sept. 1915.
From Fiskeribetjent Esager, Staby.

St. 11. Ringkjøbing, abt. { 56° 05' N. / 8° 14,5' E.

From the navigating channel in to Ringkjøbing Harbour and Ringkjøbing Forhavn. 5 July 1915. Taken in seine.
From Fiskeribetjent Jens Iversen, Gl. Sogn.

St. 12. Nymindegab, abt. { 55° 48,6' N. / 8° 11,4' E.

Taken in eel seine south of the dyke. 17 July 1915, by Tarben P. Tarbensen, Lønne Klint.

St. 13. Graadyb, abt. { 55° 27,2' N. / 8° 26,3' E.

Graadyb outside Esbjerg, between Nordby and Rindby, Fano. Depth 5 m. 12 July 1915. Taken in eel seine net worked with motor power.
From Fiskeribetjent P. Tæbring, Esbjerg.

St. 14. Hurup, abt. { 56° 52' N. / 10° 15,7' E.

East coast of Jutland between Limfjord and Mariager Fjord at Knarmov, North of Hurup. Depth 5 ft., grass bottom, 14 Oct. 1915. Taken in eel traps.
From Fiskeribetjent A. S. Larsen, Mariager.

St. 15. Als-Odde, abt. { 56° 43,6′ N. / 10° 21,8′ E.

1 naut. mile North of Mariager Fjord, 0,7 n. m. from shore, 1,2 n. m. true N. 50° E. from the mark at Als-Odde. Depth 2 m. Grass bottom. 19 Aug. 1915.

From Fiskeribetjent A. S. Larsen, Mariager.

St. 16. Mariager Fjord, Hadsund, abt. { 56° 43,9′ N. / 10° 06,3′ E.

Depth abt. 2 faths, mud and grass bottom. 14 Sept. 1915.

From Fiskeribetjent A. S. Larsen, Mariager.

St. 17. Mariager Fjord, Kjelstrup So, abt. { 56° 39,8′ N. / 9° 56,6′ E.

Depth abt. 5 m. Mud bottom with small white mussels and grass. 20 July 1915. Taken in eel hand-seine.

From Fiskeribetjent A. S. Larsen, Mariager.

St. 18. Mariager Fjord, Katbjerg Odde, abt. { 56° 38,9 N. / 9° 54,7′ E.

Depth abt. 7½ m. Mud bottom, with black mussels and grass. 20 July 1915. Taken in eel hand-seine.

From Fiskeribetjent A. S. Larsen, Mariager.

St. 19. Mariager Fjord, Hobro Harbour, abt. { 56° 38′ N. / 9° 48′ E.

Depth abt. 6 ft. Mud bottom. 26 Sept. 1916. Taken in eel seine.

From Fiskeribetjent A. S. Larsen, Mariager.

St. 20. Æbeltoft, abt. { 56° 12,5′ N. / 10° 40′ E.

2—4 cables' length N.W. of north harbour mouth at Æbeltoft. Depth 5—7 m. Weed bottom. 2 Jan. 1917. Taken in eel seine.

From Fisherman W. Olsen, President of Æbeltoft Fiskeriforening.

St. 21. Samsø, abt. { 55° 55′ N. / 10° 39,5′ E.

Stavnsfjorden. 17 June 1915. Taken in eel seine.

From Fiskerifoged Fr. Petersen, Langøre.

St. 22. Snoghøj, abt. { 55° 31,2′ N. / 9° 43,2′ E.

From the Fiskerhøjskole at Snoghøj. Depth 2—10 ft. *Zostera* bottom. 28 May 1915. Taken in stake nets and traps.

From Højskoleforstander, cand. mag. Andreas Otterstrøm, Snoghøj.

St. 23. Kolding Fjord, abt. { 55° 29,7' N. / 9° 31,3' E.

Innermost part of the fjord, between Strandhuse and Tved on the southern side. Depth abt. 3 m. 14 Nov. 1915. Taken in eel seine from a drift boat by Fisherman G. Didriksen, Strandhuse.

From Fiskerikontrollør W. Larsen, Nyborg.

St. 24. Fæno, abt. { 55° 28,4' N. / 9° 42,6' E.

South side of Fænø. 20 Nov. 1915. Taken in traps by Fisherman Chr. Christiansen, Skrillinge Strand.

From Fiskerikontrollør W. Larsen, Nyborg.

St. 25. Odense Fjord, abt. { 55° 28' N. / 10° 27' E.

Seden Strand 1/8 naut. mile SSW. of Vigelso. Depth abt. 1 m. 2 Aug. 1915.

From stud. mag. P. Jespersen, Kjøbenhavn.

St. 26. Halskov Rev, abt. { 55° 20,7' N. / 11° 06' E.

Abt. 100 faths. from Halskov Rev, Revkrogen. 27 Nov. 1916. Taken in stake nets.

From Brothers Jensen, Korsør.

St. 27. Agerso Sund, abt. { 55° 14,3' N. / 11° 11,8' E.

Egholm, Bøgevigen. Depth 14 ft. 16 Dec. 1915. Taken in eel stake net.

From Fiskerifoged N. Mortensen, Skjelskør.

St. 28. Skjelskør Inderfjord, abt. { 55° 14,4' N. / 11° 16,5' E.

"Out at the beginning of Inderfjorden". Depth 3 ft. 16 Dec. 1915. Taken in eel traps.

From Fiskerifoged N. Mortensen, Skjelskør.

St. 29. Skjelskør Nor, abt. { 55° 16' N. / 11° 18' E.

Abt. 1/4 mile from Skjelskør Town, innermost part of the Nor at "Fugleholm". Depth 2 m. 12 Dec. 1915.

From Fiskerifoged N. Mortensen, Skjelskør.

St. 30. Ise Fjord, Lynæs, abt. { 55° 56, N. / 11° 50,5' E.

Lynæs Sand, between Lynæs and Rørvig. Depth abt. 6 ft. Sand and grass. 17 July 1915. Taken in eel seine.

From Fiskeribetjent Frederiksen, Lynæs.

St. 31. Ise Fjord, Nakkehage, abt. { 55° 54' N. / 11° 42,2' E.

Depth 3—4 m. Grass bottom with clover and patches of sand. 28 Oct. 1915. Taken in eel seine.

From Proprietær N. Nielsen, Hov Vig.

St. 32. Ise Fjord, Ourø, ca. { 55° 45,7' N. / 11° 45,9' E.

Udby Vig. Depth 6—18 ft. 14 Decr. 1915. Taken in eel seine.

From Proprietær N. Nielsen, Hov Vig.

St. 33. Roskilde Fjord, Frederikssund, abt. { 55° 50,8' N. / 12° 02,5' E.

Lunderenden on the west side of the fjord, abt. 400 yards N. of Kronprins Frederiks Bridge. Depth 1—9 ft., soft bottom with mussels and weed. 1—9 Oct. 1915. Taken in eel traps.

From Brofoged H. O. Jensen, Frederikssund.

St. 34. Roskilde Fjord, Langholm, abt. { 55° 41,7' N. / 11° 58.3' E.

Depth 3.8 m. Mud with eel-grass and small white shells. 27 Oct. 1915. Taken in eel seine.

From Fiskeribetjent Frederiksen, Lynæs.

St. 35 Snekkersten, abt. { 56° 05' N. / 12° 35,5' E.

29 Nov. 1916.

From Vilh. Hansen, Snekkersten.

St. 36. Tuborg, abt. { 55° 43,7' N. / 12° 35,5' E.

Depth 10 ft. 16 June 1915.

From Fisherman P. Poulsen, Kjøbenhavn.

St. 37. Kjøbenhavn, abt. { 55° 38' N. / 12° 32' E.

Kalvebodstrand from the weir and 1 n. m. south of same. Depth 13—15 ft. 27 June 1915. Taken in eel seine.

From Capt. G. Hansen.

St. 38. Vordingborg, abt. { 54° 59' N. / 11° 57,4' E.

Between entrance to Vordingborg Harbour and to Ny Farvand N. of Færgestrømmen. Depth 6—7 ft., soft and hard bottom. 16 and 24 March 1915. Taken in eel seine.

From Fiskeribetjent R. Christiansen, Masnedø.

St. 39. Guldborg, abt. { 54° 53,7' N. / 11° 42,3' E.

Abt. 1 km. true east of Vigsnæs. Depth abt. 4 m. 20 Oct. 1915. Taken in eel seine.

From Fiskeribetjent R. Christiansen, Masnedø.

St. 40. Nykjobing (Falster), abt. { 54° 45,2' N. / 11° 51,7' E.

Coast of Falster, from Nykjøbing Harbour and abt. 1 km. north of same. Depth 1—1¼ m. 27 Oct. 1915. Taken in traps by Fisherman V. Jansen, Nykjøbing F.

From Fiskeribetjent R. Christiansen, Masnedø.

St. 41. Gedsergaard, abt. { 54° 37' N. / 11° 53' E.

Between Gedsergaard and Skjelby. Depth 12—14 ft. 8 Nov. 1915. Taken in eel traps.

From Fiskeribetjent R. Christiansen, Masnedø.

St. 42. Nakskov Fjord, abt { 54° 50,5' N. / 11° 03,3' E.

West side of Slotø. Depth 8 ft. 2 Jan. 1917. Taken in eel seine.

From Fisherman Ole Petersen, Nakskov.

St. 43. Vognsbjerg, abt. { 54° 44,4' N. / 10° 43,3' E.

East side of Langeland, 0.5 n. m. N. of Kjelds Nor Light. Depth 6—7 faths. 28 Dec. 1916. Taken on hook.

From Th. Hansen, Vognsbjerg.

St. 44. Rudkjøbing, abt. { 54° 57,5' N. / 10° 41' E.

North side of Siø, 2 n. m. from Rudkjøbing. Depth abt. 2 m. Sandy bottom with some eel grass. 26 April 1915. Taken in shrimp traps.

From S. Boesgaard, Rudkjøbing.

St. 45. Bornholm, abt. { 55° 06′ N. / 14° 42′ E.

Close to Rønne Harbour. April and May 1915.
From Fiskeribetjent V. Kristiansen, Rønne.

St. 46. Kiel Fjord, abt. { 54° 25′ N. / 10° 13,5′ E.

Laboe, at the mouth of the fjord. April 1915. Taken in shore seine.
From Professor E. Ehrenbaum, Hamburg.

St. 47. Mouth of Slien, abt. { 54° 40,5′ N. / 10° 04′ E.

12 April 1915. Taken on hook.
From Professor E. Ehrenbaum, Hamburg.

St. 48. Slesvig Bredning, abt. { 54° 31′ N. / 9° 36′ E.

The great Bredning near Slesvig town. 22—24 June 1915. Taken by Fisherman Detleff Nanz, Slesvig.
From Professor E. Ehrenbaum, Hamburg.

St. 49. Lysekil, abt. { 58° 16′ N. / 11° 26′ E.

Gåsö, right outside the mouth of Gullmarsfjord, abt. SSW. of Lysekil, in shallow water, down to 4 faths. depth. 5—10 July 1916. Taken in small fine-meshed eel traps.
From Dr. K. A. Andersson, Fiskbäckskil, Sweden.

St. 50. "Gullmarsfjorden"; precise locality not stated. 5 Nov. 1915.
From Dr. K. A. Andersson, Fiskbäckskil, Sweden.

St. 51. Stockholm, abt. { 59° 18,5′ N. / 18° 26,5′ E.

Skeppsdal, Åkersberga in the inner Skærgaard of Stockholm. 30 Nov. 1914.
From Dr. O. Nordqvist, Stockholm.

St. 52. Hudiksvall, abt. { 61° 33,6′ N. / 17°·26′ E.

Outer Skærgaard of Hudiksvall, north side of Agö. Depth 5—10 faths. Stony bottom. Summer and autumn 1915.
From Fiskeriinstruktör Hjalmar Skoglund, Hudiksvall, at the instigation of Dr. O. Nordqvist, Stockholm.

St. 53. Memel, abt. { 55° 47' N. / 21° 05' E.

Depth 10—15 m., somewhat stony bottom. 22—23 May 1916.

From Kgl. Fischmeister Siebold, Memel, at the instigation of Professor E. Ehrenbaum, Hamburg.

St. 54. Büsum, abt. { 54° 08' N. / 8° 40' E.

Mouth of the Elbe at Büsum, Ditmarsken 10—17 April 1915. Taken in shrimp trawl.

From Professor E. Ehrenbaum, Hamburg.

St. 55. Texel, abt. { 53° 08' N. / 4° 56' E.

Northern Zuider Zee, near Texel. 12—18 June 1916. Taken in traps.

From Dr. H. C. Redeke, Helder, Holland.

St. 56. Cullercoats, abt. { 55° 04' N. / 1° 27' W.

"Between tide-marks". Summer 1916.

From Professor A. Meek, Newcastle.

St. 57. Anstruther, abt. { 56° 11' N. / 2° 49' W.

26 July 1916.

From Dr. T. Wemyss Fulton, Aberdeen.

St. 58. Aberdeen, abt. { 57° 09' N. / 2° 03' W.

Bay of Nigg. 1915—1916.

From Dr. T. Wemyss Fulton, Aberdeen.

St. 59. Wick, abt. { 58° 26' N. / 3° 09' W.

29 May 1916.

From Dr. T. Wemyss Fulton, Aberdeen.

St. 60. Porsanger Fjord, abt. { 70° 30' N. / 25° 10' W.

On the shore, in eel seine. 24 Aug. 1900.

From Mag. sc. Einar Koefoed, Bergen.

St. 61. Drøbak, abt. { 59° 39,5' N. / 10° 38' E.

Christiania Fjord, near Drøbak. 18 June 1915. Taken in shallow water, seine.

From Konservator H. Kiær, Drøbak, at the instigation of Professor H. H. Gran, Christiania.

Material received since revision of text.

St. 62. Frederikshavn (Kattegat), abt. { 57° 24,5' N. / 10° 32,5' E.

Bangsbo Strand, abt. 200 m from shore. Depth 4 m. 10 May 1917. Taken on hooks baited with *Arenicola*.

From Fiskeriassistent N. Krogh, Frederikshavn.

St. 63. Randers Fjord (Kattegat), abt. { 56° 33,6' N. / 10° 14,8' E.

North end of Kareholme. Depth 2½ m. In eel traps. 26 April 1917.

From Fiskeribetjent C. Skaanning, Randers.

St. 64. Norsminde Fjord (Samsø Sea), abt. { 56° 01' N. / 10° 14,5' E.

Off Norslund, abt. 50 m. from shore. Depth 3 m. Mud bottom. 17 Decr. 1916. Taken in eel hand seine.

From Fisherman Chr. Mikkelsen, Norsminde.

St. 65. Avedøre (The Sound), abt. { 55° 37,3' N. / 12° 30,6' E.

Depth 1—3 m. Weed and sandy bottom. 1—7 May 1917. In stake net.

From Brothers Jensen, fishermen, Avedøre.

St. 66. Faxe Bay (Eastern Baltic), abt. { 55° 11' N. / 12° 07,3' E.

Off Strandegaards Dyrehave. Depth abt. 6 m. Hard sandy bottom with patches of weed. 20—29 May 1917. In stake net.

From Fisherman Jacob Nielsen, Præstø.

XI. Tables showing results of population analyses.

In the following tables are shown the results of the population analyses, of which, however, only one analysis from each locality, even where several were made. In explanation of the tables, the following should be noted.

Each »Station« is given a serial number, whereby its position may be readily ascertained from the lists in Section X, p. 351 and by means of the charts Pl. I and II. The stations are, moreover, arranged according to the waters to which they belong.

The tables give detailed information as to the number of Vertebræ (Vert), Hard rays (D_2), Pigment spots (D_1) and Rays in right pectoral fin (Pd), each character being noted separately.

In the tables for Pectoral fin rays, there will also be found noted the percentage of males (♂♂) in each sample, where the samples consisted of specimens sufficiently large for determination of sex. Our reason for noting the percentage of males in the pectoral fin lists especially is that the character in question was determinable for almost every specimen in a given sample, whereas in the case of the other characters, several specimens had as a rule to be discarded as unfit for purposes of counting, owing to uncertainty in the determination, damage, or the like.

The figures in the extreme left hand column on each page indicate the scale of variates. In the remaining columns, the number of different variates is noted for each station, either the total number, or, in parentheses, the number of males (♂♂).

There will further be found the total number of specimens in each population sample (n), the average (a) of all measurements, the Standard Deviation (σ) and the Fluctuation of average (Fl.). For calculation of σ and Fl. the following formulæ were employed:

$$\sigma = \pm \sqrt{\frac{\Sigma a p^2}{n - 1} - b^2}$$

$$Fl. = \pm \frac{\sigma}{\sqrt{n}} \cdot 0.67449 \cdot 5 .$$

No. of vertebræ	1. Norwegian sea	2. North Sea (western)				3. North Sea (eastern)						
	60	**56**	**57**	**58**	**59**	**55**	**54**	**13**	**12**	**11**	**10**	**9**
	Norway	England	Scotland	Scotland	Scotland	Holland	Germany	Denmark	Denmark	Denmark	Denmark	Denmark
	Porsanger Fjord	Cullercoats	Anstruther	Aberdeen	Wick	Texel	Büsum	Graadyb	Nymindegab	Ringkjøbing	Nissum Fjord	Nissum Bredning
126												
125												
124												
123												
122												
121												
120		1	2									
119		1	3	19				1				
118		4	20	35	1			1 (1)				
117	1	2	34	60	3		1 (1)	1 (1)				
116		8	27	67	5	1 (1)	4 (2)	5	1		2 (2)	1 (1)
115	2	6	29	41	11	3 (2)	7 (5)	8 (6)		1 (1)		6 (3)
114	2	1	16	12	21	7 (5)	26 (16)	14 (5)	1 (1)	1	6 (5)	9 (3)
113		1	3	7	6	16 (7)	33 (16)	21 (14)	1 (1)	8 (3)	11 (8)	33 (19)
112		1	2	2	2	34 (12)	56 (39)	49 (16)	7 (3)	10 (3)	21 (14)	51 (26)
111				2		31 (17)	57 (38)	54 (21)	8 (6)	15 (6)	43 (28)	36 (19)
110					1	33 (19)	42 (25)	45 (19)	12 (9)	32 (16)	49 (34)	34 (23
109				1		14 (8)	16 (11)	19 (9)	15 (8)	50 (20)	33 (18)	10 (3)
108						8 (6)	10 (6)	7 (3)	8 (4)	26 (13)	23 (11)	4 (3)
107						4 (1)	4 (3)	8 (4)	5 (3)	10 (4)	10 (4)	2 (1
106						1 (1)			2 (1)	6 (5)	3 (2)	
105						1				3 (2)	1 (1)	
104						1 (1)				3 (2)		
103												
102												
101												
n	5	25	136	246	50	154	256	233	60	165	202	186
a	**115.0**	**116.080**	**116.096**	**116.309**	**114.400**	**110.903**	**111.523**	**111.322**	**109.683**	**109.267**	**110.139**	**111.532**
σ	$\pm$ 1.225	$\pm$ 1.801	$\pm$ 1.549	$\pm$ 1.605	$\pm$ 1.401	$\pm$ 1.927	$\pm$ 1.836	$\pm$ 2.022	$\pm$ 1.911	$\pm$ 1.917	$\pm$ 1.837	$\pm$ 1.601
Fl.	$\pm$ 1.847	$\pm$ 1.214	$\pm$ 0.448	$\pm$ 0.345	$\pm$ 0.668	$\pm$ 0.524	$\pm$ 0.387	$\pm$ 0.447	$\pm$ 0.832	$\pm$ 0.503	$\pm$ 0.436	$\pm$ 0.396

No. of vertebræ	4. Skagerak			5. Cattegat									
	61 Norwegen Drøbak	49 Sweden Lysekil	50 Sweden "Gullmars Fjord"	1 Læsø	14 Hurup	2 Limfjord Hals	3 Limfjord East of Aalborg	4 Limfjord Nibe Bredning	5 Limfjord Aggersund	6 Limfjord Hjarbæk Fjord	7 Limfjord East side of Mors	8 Limfjord Sallingsund	9 Limfjord Nissum Bredning
126						1 (1)							
125													
124													
123													
122				1	4 (2)	4 (1)							
121				7 (5)	6 (1)	9 (8)							
120	5 (1)			13 (4)	13 (3)	21 (12)	1						
119	10 (3)	2		31 (19)	30 (14)	31 (18)	1 (1)						
118	17 (8)	8 (5)		41 (22)	38 (12)	28 (17)	1						
117	29 (19)	11 (4)	3 (1)	42 (23)	42 (15)	29 (14)	4 (2)		1		1 (1)		
116	19 (9)	8 (3)	1 (1)	39 (22)	27 (11)	34 (21)	10 (5)				2		1 (1)
115	9 (4)	16 (10)	3 (2)	18 (8)	17 (5)	25 (15)	18 (4)	1		3 (2)	4 (1)	3	6 (3)
114	3 (1)	5 (4)	5 (4)	9 (6)	8 (4)	8 (1)	23 (8)	2 (2)	12 (4)	8 (5)	17 (8)	13 (4)	9 (3)
113	2 (1)	4 (2)	10 (5)	4 (3)	2 (1)	7 (3)	23 (12)	8 (5)	17 (8)	13 (7)	27 (16)	25 (7)	33 (19)
112			6 (4)	1 (1)		2 (1)	24 (9)	21 (14)	41 (15)	33 (20)	53 (21)	41 (9)	51 (26)
111			3		3 (1)	2 (1)	33 (11)	20 (11)	43 (13)	56 (27)	46 (18)	52 (11)	36 (19)
110						2	29 (15)	44 (22)	46 (14)	49 (24)	25 (10)	39 (6)	34 (23)
109			1			2	16 (8)	46 (27)	26 (4)	32 (18)	15 (6)	19 (2)	10 (3)
108						1 (1)	7 (2)	22 (10)	16 (3)	11 (3)	8 (3)	5 (2)	4 (3)
107							3 (1)	14 (7)	6	5 (2)	2	1	2 (1)
106								3		1	1	1 (1)	
105										1			
104								2					
103										1 (1)			
102													
101													
n	94	54	32	206	190	206	193	183	208	213	201	199	186
a	**116.979**	**115.907**	**113.313**	**117.252**	**117.368**	**117.102**	**112.114**	**109.689**	**110.745**	**110.577**	**111.488**	**111.206**	**111.532**
σ	± 1.527	± 1.587	± 1.840	± 1.831	± 1.951	± 2.624	± 2.462	± 1.808	± 1.745	± 1.748	± 1.775	± 1.587	± 1.601
Fl.	± 0.531	± 0.728	± 1.096	± 0.430	± 0.477	± 0.617	± 0.598	± 0.451	± 0.408	± 0.404	± 0.422	± 0.379	± 0.396

No. of vertebræ	5. Cattegat										
	14 Hurup	**15** Mariager Fjord Als-Odde	**16** Mariager Fjord Hadsund	**17** Mariager Fjord Kjelstrup Sø	**18** Mariager Fjord Katbjerg Odde	**19** Mariager Fjord Hobro Harbour	**30** Ise Fjord Lynæs	**31** Ise Fjord Nakkehage	**32** Ise Fjord Ourø	**33** Roskilde Fjord Frederiks-sund	**34** Roskilde Fjord Langholm
126											
125											
124											
123			1 (1)								
122	4 (2)	2 (2)									
121	6 (1)	3 (3)	1				1				
120	13 (3)	8 (8)					1		1		
119	30 (14)	13 (11)	1				5 (2)	2	1 (1)		
118	38 (12)	17 (16)	2				5 (3)	1 (1)	12 (4)		
117	42 (15)	27 (22)	5 (2)				21 (11)	11 (1)	26 (7)		
116	27 (11)	27 (25)	9 (3)				29 (13)	14 (4)	58 (31)		
115	17 (5)	34 (30)	4 (1)	1			49 (25)	31 (12)	108 (51)	2	
114	8 (4)	22 (20)	6 (3)	3 (1)	1	1 (1)	53 (17)	42 (16)	171 (81)	2	
113	2 (1)	19 (16)	8 (1)	11 (3)	5 (1)	4 (2)	37 (21)	41 (11)	193 (89)	5	1 (1)
112		11 (11)	23 (6)	27 (8)	12 (5)	11 (5)	39 (13)	48 (11)	171 (73)	11 (3)	6 (3)
111	3 (1)	6 (6)	21 (7)	39 (11)	38 (15)	27 (14)	16 (8)	28 (1)	116 (57)	32 (6)	20 (5)
110		7 (5)	30 (14)	47 (20)	40 (21)	43 (25)	3 (1)	11 (6)	61 (27)	35 (13)	39 (18)
109		2 (1)	25 (5)	32 (14)	41 (22)	54 (22)	4 (3)	3 (1)	30 (10)	40 (7)	61 (22)
108		1	12 (4)	18 (8)	34 (15)	29 (14)	5 (2)		12 (8)	25 (8)	83 (32)
107			5 (1)	8 (3)	20 (6)	17 (11)		1	4 (2)	12 (1)	48 (16)
106			6	3	6 (1)	4 (1)			1	10 (2)	35 (11)
105			2		3 (1)	3 (1)				2 (1)	18 (7)
104				1	3 (1)	1				1 (1)	5 (2)
103			2 (2)								3 (2)
102											2 (1)
101											2 (2)
n	190	199	163	190	203	194	268	233	965	177	323
a	**117.368**	**115.427**	**110.988**	**110.174**	**109.286**	**109.304**	**114.011**	**113.223**	**112.939**	**109.463**	**107.984**
σ	± 1.951	± 2.711	± 3.194	± 1.742	± 1.819	± 1.653	± 2.214	± 1.966	± 2.057	± 1.916	± 1.926
Fl.	± 0.477	± 0.648	± 0.844	± 0.426	± 0.434	± 0.400	± 0.456	± 0.434	± 0.223	± 0.486	± 0.361

No. of vertebræ	6. Samsø Sea			7. Little Belt			8. Western Baltic			9. Islands S. of Funen
	20 Æbeltoft	**21** - Samsø	**25** Odense Fjord	**22** Snoghoj	**23** Kolding Fjord	**24** Fænø	**46** Germany Kiel Fjord	**47** Germany Slien's mouth	**48** Germany Slesvig Bredning	**44** Rudkjøbing
126										
125										
124	1	1								
123	2 (2)			2 (1)	1			2 (1)		
122	1	1		4	2 (1)	2				1
121	6 (2)	9 (5)		10 (3)	1	5 (1)	5 (2)	8 (2)		
120	18 (5)	15 (7)		40 (12)	6 (1)	19 (9)	7 (5)	13 (4)		
119	32 (12)	16 (8)		71 (26)	17 (10)	37 (5)	16 (4)	18 (4)		11 (4)
118	40 (10)	33 (18)		93 (33)	24 (11)	50 (13)	26 (8)	24 (6)		29 (16)
117	46 (13)	31 (14)		87 (27)	36 (14)	66 (24)	19 (5)	19 (6)	3 (1)	39 (7)
116	29 (7)	22 (13)		59 (26)	54 (27)	49 (15)	12 (3)	8 (3)	5 (1)	56 (18)
115	13 (5)	21 (12)		23 (12)	42 (19)	31 (11)	9 (5)	6	21 (8)	56 (18)
114	12 (4)	13 (7)	1 (1)	11 (5)	21 (7)	8 (2)	3 (1)	1	36 (18)	32 (12)
113	1	9 (6)	3	5 (4)	14 (8)	1	2		39 (11)	22 (12)
112		6 (2)	2 (1)		13 (7)	1			25 (8)	8 (5)
111		2 (2)	3		1				30 (12)	1
110			1	1 (1)					9 (2)	1 (1)
109			1 (1)						4 (1)	
108									2 (1)	
107		1 (1)								
106										
105										
104										
103										
102										
101										
n	201	180	11	406	232	269	99	99	174	256
a	**117.552**	**116.817**	**111.727**	**117.645**	**116.000**	**117.249**	**117.525**	**118.212**	**112.810**	**115.637**
σ	± 1.892	± 2.516	± 1.507	± 1.777	± 2.093	± 1.693	± 1.787	± 1.775	± 1.778	± 1.801
Fl.	± 0.450	± 0.632	± 1.532	± 0.298	± 0.464	± 0.348	± 0.606	± 0.602	± 0.454	± 0.380

No of vertebræ	10. Great Belt				11. Langeland Belt		12. Smaaland Sea			
	26	27	28	29	43	42	38	39	40	41
	Halskov Rev	Agersø Sund	Skjelskor Inderfjord	Skjelskor Nor	Vognsbjerg	Nakskov Fjord	Vordingborg	Guldborg	Nykjøbing Falster	Gedsergaard
126										
125										
124										
123	1									
122	3 (1)					1				1 (1)
121	13 (6)			1	1 (1)	1 (1)	2	1	3 (1)	5 (3)
120	27 (7)	5 (1)	1 (1)		5	5 (4)	7 (2)	5 (1)	2 (1)	3 (1)
119	53 (21)	11 (2)	6 (5)	1 (1)	1	8 (4)	23 (14)	17 (10)	21 (7)	19 (5)
118	61 (16)	16 (9)	7 (4)	4 (1)	2 (1)	14 (3)	45 (15)	25 (12)	31 (6)	31 (7)
117	38 (11)	19 (8)	11 (5)	10 (3)		23 (14)	49 (25)	53 (18)	46 (21)	48 (8)
116	33 (12)	8 (2)	14 (8)	12 (7)	1 (1)	23 (11)	97 (42)	61 (27)	50 (21)	58 (9)
115	23 (9)	13 (4)	14 (8)	26 (11)		13 (4)	73 (35)	61 (25)	63 (32)	47 (7)
114	6 (3)	5 (2)	15 (10)	38 (12)		5 (2)	40 (15)	39 (16)	24 (13)	23 (2)
113	1 (1)	1	9 (6)	42 (23)		1 (1)	20 (11)	16 (9)	11 (4)	10 (1)
112		1 (1)	11 (8)	34 (19)		2 (1)	5 (2)	5 (4)	3 (1)	4 (1)
111			2 (2)	25 (13)			1 (1)	1 (1)		
110			4 (1)	19 (7)						
109			1 (1)	3				1		
108				1						1
107				1 (1)						
106										
105										
104										
103										
102										
101										
n	259	79	95	217	10	96	362	285	254	250
a	**117.849**	**116.937**	**114.853**	**113.166**	**119.2**	**116.750**	**116.022**	**115.860**	**116.150**	**116.252**
σ	± 1.809	± 1.796	± 2.438	± 2.171	± 1.477	± 1.815	± 1.758	± 1.801	± 1.740	± 1.894
Fl.	± 0.379	± 0.681	± 0.844	± 0.497	± 1.575	± 0.625	± 0.311	± 0.360	± 0.368	± 0.404

No. of vertebræ	13. The Sound			14. Eastern Baltic			15. Gulf of Bothnia
	37 Copenhagen	**36** Tuborg	**35** Snekkersten	**45** Denmark Bornholm	**53** Germany Memel	**51** Sweden Stockholm	**52** Sweden Hudiksvall
126							
125							
124							
123							
122				3 (1)	2		
121				4 (1)	14 (4)		
120	10 (5)	1	1	25 (8)	29 (22)		2
119	17 (7)	5 (2)	3 (1)	46 (13)	47 (22)	1	5
118	30 (12)	9 (5)	3 (1)	66 (22)	31 (14)	2	14 (3)
117	47 (31)	7 (2)	8 (5)	54 (22)	24 (12)		25 (5)
116	44 (22)	10 (5)	3 (1)	22 (5)	8 (3)	1	31 (7)
115	39 (22)	7 (2)	2 (2)	12 (3)		3	18 (8)
114	24 (12)	8 (2)		4 (1)			4 (1)
113	14 (7)	2 (1)	2 (1)				3
112	4 (3)			1			
111	1 (1)		1 (1)				
110			2				
109							
108							
107							
106							
105							
104							
103							
102							
101							
n	230	49	25	237	155	7	102
a	**116.209**	**116.306**	**116.080**	**117.848**	**118.742**	**116.571**	**116.402**
σ	± 1.892	± 1.806	± 2.738	± 1.560	± 1.377	± 1.734	± 1.416
Fl.	± 0.421	± 0.870	± 1.847	± 0.341	± 0.373	± 2.211	± 0.473

The material noted on this page was received after revision of text.

No. of vertebræ (Vert) in *Zoarces viviparus*.

No. of vertebræ	Cattegat		Samsø Sea	The Sound	Eastern Baltic
	62 Frederiks-havn	**63** Randers Fjord	**64** Norsminde Fjord	**65** Avedore	**66** Faxe Bay
126					
125					
124	1		1		
123	1			1 (1)	
122	4 (1)		2 (1)		1
121	7 (1)		6 (1)		3 (1)
120	23 (10)	1 (1)	16 (7)	3 (1)	13 (5)
119	35 (12)	4 (2)	35 (11)	22 (16)	18 (5)
118	41 (15)	13 (10)	49 (15)	29 (16)	15 (4)
117	46 (21)	28 (20)	47 (19)	39 (19)	42 (15)
116	22 (8)	39 (18)	35 (10)	39 (24)	30 (13)
115	13 (5)	51 (22)	17 (4)	41 (22)	19 (6)
114	4 (2)	43 (28)	8 (3)	28 (20)	6 (4)
113	2	32 (18)	1 (1)	11 (8)	1 (1)
112		24 (7)	1	3 (2)	2 (2)
111		8 (4)		1	
110		2 (1)		1	
109					
108					
107					
106					
105					
104					
103					
102					
101					
n	199	245	218	218	150
a	**117.869**	**114.731**	**117.486**	**116.133**	**117.010**
σ	$\pm$ 1.850	$\pm$ 1.926	$\pm$ 1.787	$\pm$ 1.929	$\pm$ 1.831
Fl.	$\pm$ 0.442	$\pm$ 0.415	$\pm$ 0.408	$\pm$ 0.441	$\pm$ 0.504

No. of hard rays	1. Norwegian Sea	2. North Sea (western)				3. North Sea (eastern)						
	60 Norway Porsanger Fjord	**56** England Cullercoats	**57** Scotland Anstruther	**58** Scotland Aberdeen	**59** Scotland Wick	**55** Holland Texel	**54** Germany Büsum	**13** Denmark Graadyb	**12** Denmark Nymindegab	**11** Denmark Ringkjøbing	**10** Denmark Nissum Fjord	**9** Denmark Nissum Bredning
17												
16												
15												
14												
13												
12												
11				1								
10			6	10			1 (1)	3 (3)	1 (1)	1 (1)	5 (5)	4 (4)
9		1	16	17	1	2 (1)	2 (1)	13 (5)	1 (1)	4 (1)	9 (6)	12 (6)
8	2	11	39	56	5	13 (9)	17 (13)	28 (13)	9 (7)	24 (12)	34 (18)	24 (13)
7	3	6	41	63	10	29 (17)	18 (13)	57 (23)	12 (5)	34 (15)	62 (44)	49 (20)
6		4	24	42	11	47 (21)	32 (19)	41 (15)	15 (7)	48 (24)	38 (21)	53 (24)
5		3	13	11	14	31 (13)	17 (10)	25 (9)	16 (11)	25 (12)	22 (15)	22 (14)
4		1	2	2	7	20 (13)	17 (12)	11 (3)	7 (4)	13 (8)	12 (7)	5 (3)
3					1	10 (3)	4 (4)	4 (1)	2	2 (1)	8 (5)	
2						4 (3)	1 (1)	2 (1)		1	1	1
1												
0												
n	5	26	141	202	49	156	109	184	63	152	191	170
a	**7.4**	**7.000**	**7.234**	**7.267**	**5.837**	**5.641**	**5.954**	**6.527**	**6.016**	**6.243**	**6.539**	**6.665**
σ	+ 0.583	± 1.265	+ 1.319	+ 1.261	± 1.365	± 1.490	+ 1.545	± 1.515	+ 1.475	+ 1.372	+ 1.559	+ 1.333
Fl.	± 0.879	± 0.837	+ 0.375	± 0.299	+ 0.658	± 0.402	± 0.499	± 0.377	± 0.627	+ 0.375	± 0.381	± 0.345

No. of hard rays	4. Skagerak			5. Cattegat									
	61 Norway Drøbak	**49** Sweden Lysekil	**50** Sweden "Gullmars Fjord"	**1** Læsø	**14** Hurup	**2** Limfjord Hals	**3** Limfjord East of Aalborg	**4** Limfjord Nibe Bredning	**5** Limfjord Agger-sund	**6** Limfjord Hjarbæk Fjord	**7** Limfjord East side of Mors	**8** Limfjord Salling-sund	**9** Limfjord Nissum Bredning
17				»						»	»		
16				»						»	»		
15				»						»	»		
14				»						»	»		
13				»		1 (1)				»	»		
12				»	15 (7)	8 (3)				»	»		
11				»	23 (8)	30 (19)	1			»	»	2	
10				»	46 (13)	44 (23)	9 (3)	3 (3)	2 (2)	»	»	3	4 (4)
9	6 (3)			»	54 (17)	62 (36)	37 (17)	13 (8)	18 (8)	»	»	23 (7)	12 (6)
8	14 (8)	4 (3)	1	»	37 (17)	35 (13)	44 (21)	36 (19)	39 (14)	»	»	42 (8)	24 (13)
7	16 (5)	10 (5)		»	15 (5)	20 (13)	54 (15)	45 (26)	63 (18)	»	»	61 (12)	49 (20)
6	33 (18)	11 (6)	3 (3)	»	2	3 (1)	19 (9)	51 (19)	51 (9)	»	»	44 (7)	53 (24)
5	9 (4)	13 (6)		»	4 (1)	1	14 (7)	12 (8)	26 (3)	»	»	15 (6)	22 (14)
4	4 (1)	6 (3)		»	1		7 (3)	6 (1)	7 (1)	»	»	1	5 (3)
3	3 (2)	3 (3)		»			1	1 (1)	4 (3)	»	»		
2	1 (1)	2 (1)	1	»						»	»		1
1		2 (1)	2 (1)	»						»	»		
0		5 (2)	24 (13)	»						»	»		
n	86	56	31	»	197	204	186	167	210	»	»	191	170
a	**6.372**	**4.857**	**0.968**		**9.208**	**9.216**	**7.414**	**6.844**	**6.719**	»		**7.147**	**6.665**
σ	± 1.464	± 2.255	± 2.235	»	± 1.546	± 1.405	± 1.491	± 1.301	± 1.375	»	»	± 1.257	± 1.333
Fl.	± 0.532	± 1.016	± 1.354	»	± 0.372	± 0.332	± 0.369	± 0.340	± 0.320	»	»	± 0.307	± 0.345

No. of hard rays	5. Cattegat										
	14 Hurup	**15** Mariager Fjord Als-Odde	**16** Mariager Fjord Hadsund	**17** Mariager Fjord Kjelstrup Sø	**18** Mariager Fjord Katbjerg Odde	**19** Mariager Fjord Hobro Harbour	**30** Ise Fjord Lynæs	**31** Ise Fjord Nakkehage	**32** Ise Fjord Ourø	**33** Roskilde Fjord Frederiks-sund	**34** Roskilde Fjord Langholm
17											
16											
15											
14											
13		1 (1)									
12	15 (7)	5 (4)	1						1 (1)		
11	23 (8)	19 (18)	3 (2)				2 (2)		1		
10	46 (13)	42 (38)	10 (3)	2	2			4	7 (6)		
9	54 (17)	40 (36)	15 (4)	14 (7)	9 (3)	8 (3)	7 (5)	18 (4)	32 (20)	6	3 (1)
8	37 (17)	47 (40)	36 (13)	42 (12)	27 (15)	30 (14)	23 (12)	30 (8)	72 (38)	6	31 (12)
7	15 (5)	26 (24)	50 (17)	55 (24)	51 (21)	46 (30)	57 (27)	57 (17)	176 (90)	26 (4)	63 (21)
6	2	7 (6)	40 (11)	38 (13)	59 (26)	61 (31)	69 (30)	65 (17)	286 (122)	32 (7)	76 (31)
5	4 (1)	6 (6)	15 (5)	20 (9)	32 (12)	35 (11)	60 (24)	39 (13)	220 (97)	26 (8)	83 (29)
4	1	1	2	7 (3)	14 (6)	8 (5)	26 (7)	18 (4)	98 (42)	22 (7)	34 (14)
3					2 (1)	1 (1)	6 (5)	10 (2)	33 (10)	4 (2)	19 (8)
2							3 (1)	1	9 (2)		2 (1)
1									1		
0											
n	197	194	172	178	196	189	253	242	936	122	311
a	**9.208**	**8.742**	**7.215**	**6.871**	**6.377**	**6.402**	**5.968**	**6.322**	**5.892**	**5.787**	**5.736**
σ	± 1.546	± 1.612	± 1.470	± 1.290	± 1.340	± 1.228	± 1.449	± 1.570	± 1.460	± 1.435	± 1.409
Fl.	± 0.372	± 0.390	± 0.378	± 0.326	± 0.323	± 0.301	± 0.307	± 0.340	± 0.161	± 0.438	± 0.269

No. of hard rays	6. Samsø Sea			7. Little Belt			8. Western Baltic			9. Islands S. of Funen
	20 Æbeltoft	**21** Samsø	**25** Odense Fjord	**22** Snoghøj	**23** Kolding Fjord	**24** Fænø	**46** Germany Kiel Fjord	**47** Germany Slien's mouth	**48** Germany Slesvig Bredning	**44** Rudkjøbing
17		»					»	»		»
16		»					»	»		»
15		»					»	»		»
14		»					»	»		»
13		»					»	»		»
12	1 (1)	»			1		»	»		»
11	11 (4)	»		4	3	4 (1)	»	»	1	»
10	17 (6)	»		26 (9)	15 (7)	13 (4)	»	»	3 (1)	»
9	32 (6)	»		71 (24)	34 (14)	41 (13)	»	»	6 (4)	»
8	58 (13)	»	5 (3)	117 (40)	55 (29)	65 (17)	»	»	24 (9)	»
7	39 (15)	»	4	94 (39)	64 (34)	80 (22)	»	»	39 (13)	»
6	25 (7)	»	1 (1)	61 (25)	42 (17)	40 (13)	»	»	44 (16)	»
5	8 (4)	»		16 (4)	8 (2)	12 (5)	»	»	24 (10)	»
4		»		5 (2)	6 (2)	3 (1)	»	»	8 (2)	»
3	1	»		2	2		»	»	4 (1)	»
2		»					»	»		»
1		»					»	»		»
0		»					»	»		»
n	192	»	10	396	230	258	»	»	153	»
a	**7.922**	»	**7.400**	**7.598**	**7.439**	**7.500**	»	»	**6.458**	»
σ	± 1.536	»	± 0.664	± 1.387	± 1.499	+ 1.339	»	»	+ 1.456	»
Fl.	± 0.374	»	± 0.708	± 0.235	+ 0.333	± 0.281	»	»	+ 0.397	»

No. of hard rays	10. Great Belt				11. Langeland Belt		12. Smaaland Sea			
	26 Halskov Rev	27 Agersø-Sund	28 Skjelskør Inderfjord	29 Skjelskør Nor	43 Vognsbjerg	42 Nakskov Fjord	38 Vording-borg	39 Guldborg	40 Nykjøbing Falster	41 Gedser-gaard
17							»			
16							»			
15							»			
14							»			
13						1	»	1		5 (3)
12	5 (3)	1	2 (1)		1	3 (1)	»	2 (1)	5 (1)	16 (2)
11	12 (5)	1	2 (1)	2 (1)		6 (3)	»	8 (3)	12 (3)	39 (7)
10	29 (13)	5 (1)	6 (4)	6 (3)	3 (3)	17 (6)	»	34 (14)	35 (14)	51 (9)
9	56 (21)	21 (6)	17 (9)	22 (11)	1	22 (8)	»	60 (26)	60 (26)	66 (11)
8	78 (24)	28 (13)	26 (17)	46 (20)	3 (1)	24 (12)	»	88 (33)	62 (32)	45 (11)
7	47 (11)	14 (3)	17 (10)	51 (26)		18 (11)	»	66 (33)	44 (16)	15 (2)
6	25 (7)	5 (3)	13 (6)	43 (16)	1	3 (1)	»	18 (9)	25 (13)	3
5	5 (3)		9 (7)	29 (11)	1	2 (1)	»	5 (4)	7 (4)	
4	1		2 (2)	14 (7)			»	2 (1)		
3	2			3 (1)			»	1		
2							»			
1	1						»			
0							»			
n	261	75	94	216	10	96	»	285	250	240
a	**8.142**	**8.187**	**7.628**	**6.819**	**8.600**	**8.635**	»	**8.123**	**8.284**	**9.471**
σ	± 1.595	± 1.147	± 1.673	± 1.595	± 2.075	± 1.545	»	± 1.405	± 1.519	± 1.461
Fl.	± 0.333	± 0.447	± 0.582	± 0.366	± 2.213	± 0.532	»	± 0.281	± 0.324	± 0.318

No. of hard rays	13. The Sound			14. Eastern Baltic			15. Gulf of Bothnia
	37 Copenhagen	**36** Tuborg	**35** Snekkersten	**45** Denmark Bornholm	**53** Germany Memel	**51** Sweden Stockholm	**52** Sweden Hudiksvall
17	»	»					1
16	»	»					
15	»	»					3
14	»	»		1 (1)	1		3 (1)
13	»	»	1	1 (1)	9 (4)	3	7 (1)
12	»	»		6 (2)	32 (8)	1	17 (1)
11	»	»		29 (9)	49 (29)	1	26 (9)
10	»	»	2 (1)	45 (17)	37 (22)	3	23 (8)
9	»	»	8 (4)	49 (16)	19 (11)		12 (4)
8	»	»	6 (5)	26 (6)	5 (2)		1
7	»	»	4 (1)	7 (3)			
6	»	»	1 (1)	1			
5	»	»	1				
4	»	»	1 (1)				
3	»	»					
2	»	»					
1	»	»					
0	»	»					
n	»	»	24	165	152	8	93
a	»	»	**8.167**	**9.527**	**10.757**	**11.500**	**11.086**
σ	»	»	± 1.786	± 1.291	± 1.225	± 1.427	± 1.599
Fl.	»	»	± 1.229	± 0.339	± 0.335	± 1.702	± 0.559

The material noted on this page was received after revision of text.

No. of hard rays (D_2) in *Zoarces viviparus*.

No. of hard rays	Cattegat		Samsø Sea	The Sound	Eastern Baltic
	62	**63**	**64**	**65**	**66**
	Frederiks-havn	Randers Fjord	Norsminde Fjord	Avedøre	Faxe Bay
18					
17					
16					
15					
14					1
13	3 (1)			4	2 (1)
12	12 (2)		1	24	11 (3)
11	25 (9)	7 (2)	7 (3)	54	26 (10)
10	61 (22)	20 (11)	30 (10)	76	45 (16)
9	53 (20)	56 (38)	34 (11)	103	42 (17)
8	32 (15)	72 (37)	55 (12)	66	28 (10)
7	19 (8)	67 (33)	56 (18)	25	5 (5)
6	5 (3)	16 (9)	26 (12)	7	
5		4 (3)	9 (4)		
4		1			
3			1		
2					
1					
0					
n	210	243	219	359	160
a	**9.352**	**8.008**	**7.904**	**9.376**	**9.656**
σ	$\pm$ 1.458	$\pm$ 1.256	$\pm$ 1.511	$\pm$ 1.448	$\pm$ 1.338
Fl.	$\pm$ 0.339	$\pm$ 0.272	$\pm$ 0.344	$\pm$ 0.258	$\pm$ 0.357

No. of pigment spots	1. Norwegian Sea	2. North Sea (western)				3. North Sea (eastern)						
	60 Norway Porsanger Fjord	**56** England Cullercoats	**57** Scotland Anstruther	**58** Scotland Aberdeen	**59** Scotland Wick	**55** Holland Texel	**54** Germany Büsum	**13** Denmark Graadyb	**12** Denmark Nyminde-gab	**11** Denmark Ring-kjøbing	**10** Denmark Nissum Fjord	**9** Denmark Nissum Bredning
21	»											
20	»											
19	»											
18	»									1	1 (1)	
17	»					1 (1)	5 (4)	3	2	1 (1)	1	1 (1)
16	»		1	6	1	1 (1)	5 (1)	7 (2)	1	3 (1)	4 (4)	5 (3)
15	»		5	12	5	6 (4)	23 (14)	13 (4)	10 (5)	15 (10)	9 (7)	17 (8)
14	»	3	16	31	3	12 (6)	25 (14)	23 (11)	9 (7)	23 (12)	32 (21)	35 (22)
13	»	8	28	54	8	13 (6)	42 (25)	39 (19)	16 (10)	36 (19)	35 (21)	37 (28)
12	»	9	20	59	3	17 (5)	38 (26)	37 (18)	13 (11)	26 (15)	29 (17)	48 (27)
11	»	2	9	18		10 (4)	51 (31)	30 (19)	6 (2)	11 (5)	23 (13)	23 (13)
10	»		2	3		11 (7)	31 (21)	31 (12)	1 (1)	4 (1)	7 (3)	7 (3)
9	»			1		1	13 (9)	10 (5)	4 (1)	1 (1)	1 (1)	
8	»											1 (1)
7	»											
n	»	22	81	184	20	72	233	193	62	121	142	174
a	»	**12.545**	**12.815**	**12.810**	**13.650**	**12.403**	**12.232**	**12.233**	**12.935**	**13.058**	**12.796**	**12.816**
σ	»	± 0.867	± 1.209	± 1.283	± 1.191	± 1.709	± 1.896	± 1.865	± 1.814	± 1.507	± 1.552	± 1.494
Fl.	»	± 0.623	± 0.453	± 0.319	± 0.898	± 0.679	± 0.419	± 0.453	± 0.777	± 0.462	± 0.439	± 0.382

No. of pigment spots	4. Skagerak			5. Cattegat									
	61 Norway Drøbak	**49** Sweden Lysekil	**50** Sweden »Gull-mars-Fjord«	**1** Læsø	**14** Hurup	**2** Limfjord Hals	**3** Limfjord East s. of Aalborg	**4** Limfjord Nibe Bredning	**5** Limfjord Agger-sund	**6** Limfjord Hjarbæk Fjord	**7** Limfjord East side of Mors	**8** Limfjord Salling-sund	**9** Limfjord Nissum Bredning
21			»	1									
20			»										
19			»							1			
18			»	7 (2)	1		1		1	1			
17	3	1 (1)	»	14 (2)	3	4 (2)	2 (1)		1	3	3 (2)		1 (1)
16	16 (5)	1	»	14 (8)	11 (3)	10 (6)	7 (4)	4 (1)	4 (1)	10	2 (1)	1	5 (3)
15	26 (15)	9 (3)	»	26 (19)	19 (11)	23 (13)	10 (6)	11 (4)	15 (4)	16	9 (4)	15 (5)	17 (8)
14	27 (14)	13 (7)	»	48 (29)	38 (16)	38 (19)	30 (11)	29 (13)	29 (10)	34	31 (11)	19 (6)	35 (22)
13	19 (8)	12 (5)	»	33 (23)	45 (15)	35 (18)	35 (11)	40 (27)	56 (16)	42	47 (25)	26 (7)	37 (20)
12	1 (1)	7 (6)	»	31 (15)	32 (12)	40 (20)	35 (12)	53 (26)	42 (9)	39	52 (28)	40 (8)	48 (27)
11			»	9 (4)	9 (1)	17 (7)	19 (6)	25 (13)	29 (6)	14	22 (7)	29 (5)	23 (13)
10			»	4 (2)	5 (1)	7 (5)	9 (4)	11 (5)	9 (5)	4	8 (3)	10 (3)	7 (3)
9			»		3		4 (2)	3 (2)	4		4 (1)		
8			»				2						1 (1)
7			»										
n	92	43	»	187	166	174	154	176	190	164	178	140	174
a	**14.500**	**13.721**	»	**13.963**	**13.295**	**13.201**	**12.727**	**12.517**	**12.668**	**13.244**	**12.612**	**12.451**	**12.816**
σ	± 1.135	± 1.187	»	± 1.899	± 1.619	± 1.609	± 1.781	± 1.450	± 1.541	± 1.602	± 1.470	± 1.454	± 1.494
Fl.	± 0.399	± 0.611	»	± 0.468	± 0.424	± 0.411	± 0.484	± 0.368	± 0.377	± 0.422	± 0.371	± 0.415	± 0.382

No. of pigment spots	5. Cattegat										
	14 Hurup	**15** Mariager Fjord Als-Odde	**16** Mariager Fjord Hadsund	**17** Mariager Fjord Kjelstrup Sø	**18** Mariager Fjord Katbjerg Odde	**19** Mariager Fjord Hobro Harbour	**30** Ise Fjord Lynæs	**31** Ise Fjord Nakkehage	**32** Ise Fjord Ourø	**33** Roskilde Fjord Frederiks-sund	**34** Roskilde Fjord Langholm
21											
20								1			
19					1 (1)						
18	1	1 (1)	1 (1)				2 (1)	1 (1)	2 (2)	1	
17	3	3 (3)	2		2 (1)		14 (7)	1 (1)	16 (7)		1
16	11 (3)	10 (9)	5	3		4 (1)	23 (8)	8 (4)	36 (15)	2	7 (4)
15	19 (11)	15 (14)	13 (2)	9 (6)	3 (2)	6 (4)	43 (19)	40 (10)	109 (56)	17 (3)	15 (7)
14	38 (16)	39 (31)	22 (10)	12 (2)	16 (5)	15 (7)	60 (21)	59 (14)	170 (66)	22 (5)	32 (9)
13	45 (15)	38 (36)	21 (5)	39 (19)	26 (11)	37 (19)	60 (29)	47 (10)	194 (80)	45 (7)	70 (30)
12	32 (12)	24 (21)	41 (15)	37 (16)	34 (9)	45 (22)	20 (11)	43 (12)	108 (34)	41 (12)	63 (23)
11	9 (1)	16 (14)	18 (3)	26 (8)	31 (15)	24 (12)	12 (6)	7 (1)	42 (20)	19 (6)	38 (13)
10	5 (1)	8 (8)	18 (6)	35 (9)	32 (18)	17 (9)	5 (5)	4	14 (5)	9 (3)	12 (3)
9	3	4 (4)	8 (2)	9 (4)	18 (6)	20 (8)	2 (2)	1 (1)	2 (1)	4	14 (7)
8		2 (1)	2 (1)	2	6 (4)	3 (2)				1	4 (1)
7			1	1 (1)							1
n	166	160	152	173	169	171	241	212	693	161	257
a	**13.295**	**13.063**	**12.322**	**11.803**	**11.473**	**11.830**	**13.888**	**13.500**	**13.473**	**12.627**	**12.339**
σ	± 1.619	± 1.835	± 1.985	± 1.729	± 1.868	± 1.767	± 1.668	± 1.463	± 1.480	± 1.578	± 1.702
Fl.	± 0.424	± 0.489	± 0.543	± 0.443	± 0.485	± 0.456	± 0.362	± 0.339	± 0.190	± 0.419	± 0.358

No. of pigment spots	6. Samsø Sea			7. Little Belt			8. Western Baltic Sea			9. Islands S. of Funen
	20 Ebeltoft	**21** Samsø	**25** Odense Fjord	**22** Snoghøj	**23** Kolding Fjord	**24** Fænø	**46** Germany Kiel Fjord	**47** Germany Slien's mouth	**48** Germany Slesvig Bredning	**44** Rudkjøbing
21			»							
20			»						1 (1)	
19		3 (1)	»	2 (1)	1 (1)					
18	1	2	»	7 (2)	4 (4)	3	1		2	4 (2)
17	8	7 (4)	»	21 (9)	12 (6)	6 (2)	8	3	9 (3)	5
16	28 (11)	15 (6)	»	38 (15)	28 (13)	21 (8)	14	10	24 (9)	20 (9)
15	41 (14)	33 (20)	»	80 (37)	48 (17)	44 (15)	13	21	23 (12)	49 (15)
14	46 (12)	37 (22)	»	60 (25)	39 (17)	53 (14)	13	29	37 (9)	44 (17)
13	31 (10)	38 (19)	»	42 (12)	30 (18)	43 (15)	22	19	36 (16)	38 (13)
12	16 (4)	13 (5)	»	23 (10)	15 (3)	16 (4)	4	6	14 (6)	12 (4)
11	3 (2)	5 (3)	»	7	2 (1)	5 (2)	4	2	1	2
10	2		»			1			2	1 (1)
9			»							
8			»							
7			»							
n	176	153	»	280	179	192	79	90	149	175
a	**14.256**	**14.183**	»	**14.529**	**14.531**	**14.115**	**14.342**	**14.144**	**14.248**	**14.274**
σ	$\pm$ 1.480	$\pm$ 1.608	»	$\pm$ 1.586	$\pm$ 1.526	$\pm$ 1.439	$\pm$ 1.687	$\pm$ 1.286	$\pm$ 1.626	$\pm$ 1.408
Fl.	$\pm$ 0.376	$\pm$ 0.438	»	$\pm$ 0.320	$\pm$ 0.385	$\pm$ 0.350	$\pm$ 0.640	$\pm$ 0.457	$\pm$ 0.449	$\pm$ 0.359

No. of pigment spots	10 Great Belt				11. Langeland Belt		12. Smaaland Sea			
	26 Halskov Rev	**27** Agersø-Sund	**28** Skjelskør Inderfjord	**29** Skjelskør Nor	**43** Vognsbjerg	**42** Nakskov Fjord	**38** Vording borg	**39** Guldborg	**40** Nykjøbing Falster	**41** Gedser-gaard
21	1									
20	1									
19	1						1 (1)	3 (1)		
18	3 (1)	1				2 (1)	2 (2)	3 (1)	4 (1)	3 (1)
17	11 (3)	2	1	4 (2)		5 (2)	10 (6)	13 (6)	14 (8)	3
16	17 (8)	5 (2)	6 (2)	11 (6)	1	15 (8)	19 (16)	26 (13)	27 (13)	14
15	41 (16)	5 (1)	10 (6)	26 (17)	1	11 (5)	36 (26)	49 (31)	39 (17)	35 (5)
14	65 (14)	21 (8)	26 (17)	46 (9)		20 (7)	48 (42)	55 (15)	52 (23)	53 (10)
13	41 (12)	10 (3)	20 (13)	56 (25)	2 (2)	15 (6)	28 (19)	53 (27)	43 (16)	43 (9)
12	23 (13)	4 (2)	6 (5)	25 (12)	1 (1)	11 (8)	12 (7)	24 (9)	21 (6)	26 (2)
11	7 (4)	2 (1)		16 (9)		3 (1)	1 (1)	5 (3)	1	7
10			1 (1)	1		1 (1)		3 (2)	3 (1)	1
9			1 (1)							1
8										
7										
n	211	50	71	185	5	83	157	234	204	186
a	**14.142**	**14.020**	**13.775**	**13.470**	**13.800**	**14.169**	**14.395**	**14.167**	**14.250**	**13.780**
σ	± 1.630	± 1.450	± 1.336	± 1.419	± 1.691	± 1.738	± 1.431	± 1.642	± 1.582	± 1.486
Fl.	± 0.378	± 0.692	± 0.535	± 0.352	± 2.550	± 0.643	± 0.385	± 0.362	± 0.374	± 0.368

No. of pigment spots	13. The Sound			14. Eastern Baltic			15. Gulf of Bothnia
	37 Copenhagen	**36** Tuborg	**35** Snekkersten	**45** Denmark Bornholm	**53** Germany Memel	**51** Sweden Stockholm	**52** Sweden Hudiksvall
21		»					
20		»					
19	1	»					
18	6 (2)	»	2 (1)				
17	11 (6)	»	1 (1)	2 (1)			
16	25 (13)	»	2 (2)	6 (3)	6 (6)		3 (1)
15	38 (19)	»	3 (1)	25 (9)	8 (5)		1
14	67 (37)	»	4 (3)	43 (14)	25 (13)	1	12 (2)
13	44 (25)		6 (3)	38 (16)	44 (19)		25 (7)
12	15 (7)	»	2	42 (14)	26 (9)	2	30 (9)
11	3 (2)	»		29 (6)	11 (8)	1	17 (5)
10		»		18 (5)	7 (5)	2	4
9		»		3 (2)			
8		»					
7		»					
n	210	»	20	206	127	6	92
a	**14.319**	»	**14.4**	**12.772**	**12.921**	**11.500**	**12.424**
σ	± 1.496	»	± 1.820	± 1.691	± 1.392	± 1.533	± 1.269
Fl.	± 0.348	»	± 1.373	± 0.397	± 0.417	± 2.110	± 0.446

The material noted on this page was received after revision of text.

No. of pigment spots (PigmD1) in *Zoarces viviparus*.

No. of pigment spots	Cattegat		Samsø Sea	The Sound	Eastern Baltic
	62	63	64	65	66
	Frederikshavn	Randers Fjord	Norsminde Fjord	Avedøre	Faxe Bay
21					
20				1	
19	1	1		3	2
18	1 (1)	10	2 (1)	3	4 (2)
17	3 (2)	19	9 (1)	18	6 (4)
16	7 (1)	35	22 (5)	45	10 (2)
15	17 (5)	77	37 (14)	66	24 (9)
14	41 (15)	98	48 (15)	74	28 (14)
13	33 (17)	107	37 (9)	64	30 (12)
12	27 (10)	69	13 (1)	21	9 (3)
11	14 (7)	42	6 (1)	10	6 (2)
10	4 (1)	13	1 (1)	2	4 (1)
9	2 (2)	3			
8		1			
7					
n	150	475	175	307	123
a	**13.293**	**13.589**	**14.177**	**14.322**	**14.049**
σ	± 1.677	± 1.822	± 1.500	± 1.622	± 1.846
Fl.	± 0.462	± 0.282	± 0.382	± 0.312	± 0.562

No. of pectoral rays	1. Norwegian Sea	2. North Sea (western)				3. North Sea (eastern)						
	60 Norway Porsanger Fjord	**56** England Cullercoats	**57** Scotland Anstruther	**58** Scotland Aberdeen	**59** Scotland Wick	**55** Holland Texel	**54** Germany Büsum	**13** Denmark Graadyb	**12** Denmark Nymindegab	**11** Denmark Ringkjobing	**10** Denmark Nissum Fjord	**9** Denmark Nissum Bredning
22												
21										1	1	
20	1		30	5	2	4 (2)	16 (10)	13 (3)	18 (9)	36 (16)	37 (20)	43 (23)
19	4	15 (11)	99	110	20	52 (30)	128 (79)	110 (44)	39 (25)	106 (51)	132 (87)	124 (66)
18		11 (6)	14	134	29	94 (44)	118 (81)	103 (45)	12 (7)	26 (13)	34 (21)	37 (15)
17				5	3	7 (4)	8 (5)	9 (5)		3 (2)		1 (1)
16				1				1 (1)				
n	5	26	143	255	54	157	270	236	69	172	204	205
a	**19.200**	**18.577**	**19.112**	**18.443**	**18.389**	**18.338**	**18.563**	**18.530**	**19.087**	**19.035**	**19.025**	**19.020**
σ	± 0.458	± 0.517	± 0.545	± 0.592	± 0.659	± 0.606	± 0.653	± 0.680	± 0.658	± 0.675	± 0.607	± 0.642
Fl.	± 0.691	± 0.342	± 0.154	± 0.125	± 0.302	± 0.163	± 0.134	± 0.149	± 0.267	± 0.174	± 0.143	± 0.151
Proc. ♂♂.		68.0				50.9	57.4	41.5	59.4	47.7	62.7	51.2

No. of pectoral rays	4. Skagerak			5. Cattegat									
	61 Norway Drøbak	49 Sweden Lysekil	50 Sweden Gullmars Fjord	1 Læsø	14 Hurup	2 Limfjord Hals	3 Limfjord East of Aalborg	4 Limfjord Nibe Bredning	5 Limfjord Aggersund	6 Limfjord Hjarbæk Fjord	7 Limfjord East side of Mors	8 Limfjord Sallingsund	9 Limfjord Nissum Bredning
22													
21		2			2	1 (1)	8 (4)	1	2 (1)	3 (1)	7 (4)	2	
20	22 (12)	20 (10)	24 (13)	24 (13)	38 (18)	40 (22)	71 (36)	58 (28)	61 (15)	38 (22)	77 (31)	41 (11)	43 (23)
19	66 (33)	32 (19)	9 (5)	140 (73)	134 (43)	142 (79)	112 (40)	125 (69)	143 (44)	139 (71)	106 (46)	134 (28)	124 (66)
18	9 (2)	1		44 (28)	29 (11)	28 (12)	16 (4)	21 (10)	13 (2)	34 (16)	17 (7)	25 (3)	37 (15)
17	1					1 (1)							1 (1)
16													
n	98	55	33	208	203	212	207	205	219	214	207	202	205
a	**19.112**	**19.418**	**19.727**	**18.904**	**19.064**	**19.057**	**19.343**	**19.190**	**19.237**	**19.047**	**19.357**	**19.099**	**19.020**
σ	± 0.591	± 0.602	± 0.470	± 0.568	± 0.606	± 0.598	± 0.678	± 0.609	± 0.566	± 0.626	± 0.682	± 0.599	± 0.642
Fl.	± 0.201	± 0.274	± 0.276	± 0.133	± 0.143	± 0.139	± 0.159	± 0.143	± 0.129	± 0.144	± 0.160	± 0.142	± 0.151
Proc. ♂♂.	47.9	52.7	54.5	51.8	35.5	54.2	40.6	52.2	28.3	51.4	42.5	20.8	51.2

No. of pectoral rays	5. Cattegat										
	14 Hurup	**15** Mariager Fjord Als-Odde	**16** Mariager Fjord Hadsund	**17** Mariager Fjord Kjelstrup Sø	**18** Mariager Fjord Katbjerg Odde	**19** Mariager Fjord Hobro Harbour	**30** Isc Fjord Lynæs	**31** Ise Fjord Nakkehage	**32** Ise Fjord Ourø	**33** Roskilde Fjord Frederiks-sund	**34** Roskilde Fjord Langholm
22											
21	2	1 (1)	2 (1)	2 (1)	4 (1)	9 (4)	2 (1)	2 (1)	10 (5)	3	3 (1)
20	38 (18)	33 (32)	45 (11)	70 (25)	72 (37)	92 (47)	92 (36)	75 (17)	325 (145)	41 (13)	95 (36)
19	134 (43)	144 (126)	111 (38)	106 (39)	117 (43)	96 (48)	177 (80)	153 (44)	583 (277)	125 (27)	197 (79)
18	29 (11)	24 (21)	23 (7)	16 (4)	21 (11)	14 (7)	12 (9)	16 (3)	57 (17)	28 (8)	35 (10)
17		1								1	
16											
n	203	203	181	194	214	211	283	246	975	198	330
a	**19.064**	**19.044**	**19.144**	**19.299**	**19.276**	**19.455**	**19.297**	**19.256**	**19.295**	**19.086**	**19.200**
σ	± 0.606	± 0.566	± 0.634	± 0.627	± 0.660	± 0.685	± 0.556	± 0.581	± 0.588	± 0.651	± 0.626
Fl.	± 0.143	± 0.134	± 0.159	± 0.152	± 0 152	± 0.159	± 0.111	± 0.125	± 0.063	± 0.156	± 0.116
Proc. ♂♂	35.5	88.7	31.5	35.6	43.0	50.2	44.5	26.4	45.5	24.2	38.2

No. of pectoral rays	6. Samsø Sea			7. Little Belt			8. Western Baltic			9. Islands S. of Funen
	20 Æbeltoft	**21** Samsø	**25** Odense Fjord	**22** Snoghøj	**23** Kolding Fjord	**24** Fænø	**46** Germany Kiel Fjord	**47** Germany Slien's mouth	**48** Germany Slesvig Bredning	**44** Rudkjøbing
22										
21	5	4 (3)		8 (4)	6 (3)	5 (2)		1 (1)		1
20	69 (17)	49 (26)	7 (3)	196 (68)	94 (36)	110 (33)	23 (6)	30 (7)	11 (4)	80 (24)
19	112 (37)	119 (61)	3 (1)	214 (77)	126 (66)	141 (43)	68 (23)	66 (19)	102 (38)	124 (47)
18	13 (4)	11 (6)	1	18 (7)	13 (5)	14 (2)	5 (2)	2 (1)	59 (9)	22 (10)
17							2 (1)		1	2
16									2 (1)	
n	199	183	11	436	239	270	98	99	175	229
a	**19.332**	**19.251**	**19.545**	**19.445**	**19.389**	**19.393**	**19.143**	**19.303**	**18.680**	**19.245**
σ	$\pm$ 0.637	$\pm$ 0.596	$\pm$ 0.709	$\pm$ 0.607	$\pm$ 0.632	$\pm$ 0.617	$\pm$ 0.593	$\pm$ 0.524	$\pm$ 0.655	$\pm$ 0.663
Fl.	$\pm$ 0.152	$\pm$ 0.148	$\pm$ 0.721	$\pm$ 0.098	$\pm$ 0.138	$\pm$ 0.127	$\pm$ 0.202	$\pm$ 0.178	$\pm$ 0.167	$\pm$ 0.148
Proc. ♂♂.	29.1	52.4	36.4	35.8	46.0	29.6	32.7	28.3	29.7	39.7

No. of pectoral rays	10. Great Belt				11. Langeland Belt		12. Smaaland Sea			
	26 Halskov Rev	**27** Agersø-Sund	**28** Skjelskør Inderfjord	**29** Skjelskør Nor	**43** Vognsbjerg	**42** Nakskov Fjord	**38** Vording-borg	**39** Guldborg	**40** Nykjøbing Falster	**41** Gedser gaard
22										
21	5 (4)	1		3 (2)		2 (1)	3 (1)	3 (1)	5 (3)	1
20	82 (28)	22 (9)	23 (15)	54 (26)	3 (1)	31 (12)	112 (54)	77 (33)	84 (43)	39 (6)
19	160 (51)	46 (15)	61 (37)	152 (62)	7 (3)	59 (29)	237 (104)	200 (83)	155 (56)	161 (28)
18	18 (6)	8 (4)	13 (7)	14 (3)	1	8 (3)	31 (15)	22 (11)	18 (11)	47 (10)
17		1		1 (1)		1	3			2
16										
n	265	78	97	224	11	101	386	302	262	250
a	**19.279**	**19.179**	**19.103**	**19.196**	**19.181**	**19.248**	**19.210**	**19.202**	**19.290**	**18.960**
σ	± 0.614	± 0.680	± 0.603	± 0.582	± 0.606	± 0.669	± 0.625	± 0.573	± 0.619	± 0.629
Fl.	± 0.127	± 0.260	± 0.207	± 0.131	± 0.616	± 0.225	± 0.107	± 0.111	± 0.129	± 0.134
Proc. ♂♂.	33.6	35.9	60.8	42.0	36.4	44.6	45.1	42.4	43.1	17.6

No. of pectoral rays	13. The Sound			14. Eastern Baltic			15. Gulf of Bothnia
	37 Copenhagen	**36** Tuborg	**35** Snekkersten	**45** Denmark Bornholm	**53** Germany Memel	**51** Sweden Stockholm	**52** Sweden Hudiksvall
22							
21							
20	34 (18)	10 (2)	4 (2)	7 (2)	11 (7)		11 (3)
19	157 (83)	36 (17)	19 (9)	151 (50)	90 (48)	4	71 (15)
18	41 (20)	3	2 (2)	72 (23)	54 (23)	3	18 (7)
17				2	4 (1)	1	1 (1)
16							
n	232	49	25	232	159	8	101
a	**18.970**	**19.143**	**19.080**	**18.703**	**18.679**	**18.375**	**18.911**
σ	$\pm$ 0.572	$\pm$ 0.501	$\pm$ 0.494	$\pm$ 0.538	$\pm$ 0.642	$\pm$ 0.757	$\pm$ 0.574
Fl.	$\pm$ 0.127	$\pm$ 0.241	$\pm$ 0.333	$\pm$ 0.119	$\pm$ 0.172	$\pm$ 0.903	$\pm$ 0.193
Proc. ♂♂.	52.1	38.8	52.0	32 3	49.6		25.7

The material noted on this page was received after revison of text.

No. of pectoral rays (Pd og Ps) in *Zoarces viviparus*.

No. of pectoral rays	Cattegat		Samsø Sea	The Sound	Eastern Baltic
	62	63	64	65	66
	Frederiks-havn	Randers Fjord	Norsminde Fjord	Avedøre	Faxe Bay
right (Pd)					
22					
21	2 (2)		4 (2)		
20	40 (20)	101 (49)	82 (30)	31 (17)	15 (6)
19	144 (53)	131 (71)	128 (37)	144 (84)	110 (39)
18	36 (14)	16 (12)	13 (5)	52 (32)	44 (19)
17			1		2 (2)
16		1 (1)			
n	222	249	228	227	171
a	**19.054**	**19.329**	**19.329**	**18.907**	**18.807**
σ	± 0.614	± 0.633	± 0.632	± 0.603	± 0.601
Fl.	± 0.139	± 0.135	± 0.141	± 0.135	± 0.155
Proc. ♂♂	40.1	53.4	32.5	58.6	38.7
left (Ps)					
22					
21	2 (2)	6 (3)	3 (1)		
20	43 (16)	104 (53)	89 (29)	37 (26)	17 (8)
19	142 (60)	127 (67)	125 (41)	146 (83)	113 (38)
18	33 (11)	16 (13)	11 (3)	46 (26)	40 (20)
17	1	1 (1)			
16					
n	221	254	228	229	170
a	**19.054**	**19.386**	**19.368**	**18.961**	**18.865**
σ	± 0.630	± 0.660	± 0.598	± 0.604	± 0.568
Fl.	± 0.143	± 0.140	± 0.134	± 0.135	± 0.147

XII. Tables showing results of offspring analyses (decade investigations) within three selected populations.

The following tables show the results of the individual offspring analyses within three populations, *viz*: Stat. 31, Ise Fjord (1914), Stat. 33, Roskilde Fjord (1915) and Stat. 22, Little Belt (1915).

From each gravid female, 10 embryos were taken (a "decade"), in which the number of vertebræ had been determined.

The figures given in the following tables thus show the average number of vertebræ of these decades, and fluctuations in the same. The average numbers of vertebræ have for the sake of convenience been arranged in order, the highest figures first, the lowest last. The tables show that several of the average figures in the populations from Stat. 31 appear in both other populations.

A. STAT. 31. NAKKEHAGE, ISE FJORD. AUTUMN 1914.

AVERAGE NUMBERS OF VERTEBRÆ IN THE DECADES AND FLUCTUATION OF SAME.

Population: **113.42** (Fluct. ± 0.49).

a	Fl.	a	Fl.	a	Fl.
118.9 —	± 1.6	113.8 —	± 1.7	112.6 —	± 1.9
116.8 —	1.3	.8 —	1.9	.5 —	1.6
115.9 —	1.5	.8 —	1.6	.5 —	1.8
.8 —	2.1	.7 —	1.9	.5 —	0.9
.6 —	1.8	.7 —	0.9	.4 —	1.6
.4 —	1.4	.7 —	2.3	.4 —	1.3
.4 —	1.4	.7 —	1.8	.4 —	1.2
.3 —	1.7	.6 —	2.3	.4 —	1.3
.3 —	1.8	.6 —	1.3	.4 —	1.4
.2 —	1.2	.6 —	1.2	.4 —	1.7
.2 —	1.1	.5 —	1.9	.3 —	1.5
.1 —	1.3	.5 —	1.8	.3 —	1.4
.1 —	0.8	.5 —	2.1	.3 —	1.5
.0 —	1.0	.5 —	2.4	.3 —	1.9
114.9 —	1.9	.4 —	1.4	.2 —	0.8
.9 —	1.0	.4 —	1.2	.2 —	1.6
.9 —	2.5	.4 —	1.5	.2 —	2.5
.7 —	1.3	.3 —	1.8	.0 —	2.2
.7 —	1.7	.3 —	1.4	111.9 —	1.7
.6 —	1.3	.3 —	0.5	.9 —	1.1
.6 —	1.7	.3 —	1.0	.9 —	1.3
.5 —	2.5	.3 —	1.6	.8 —	2.0
.5 —	1.2	.3 —	1.3	.7 —	1.8
.5 —	1.6	.2 —	1.4	.7 —	1.5
.4 —	1.8	.2 —	1.9	.6 —	1.6
.4 —	1.2	.1 —	1.9	.5 —	1.3
.3 —	2.8	.1 —	1.6	.5 —	1.2
.3 —	1.8	.1 —	0.8	.5 —	1.4
.3 —	1.8	.1 —	1.9	.4 —	1.5
.2 —	1.6	.1 —	1.1	.3 —	0.9
.2 —	1.1	.0 —	1.8	.3 —	1.3
.1 —	1.5	.0 —	0.7	.2 —	2.3
.1 —	1.5	.0 —	1.3	.2 —	1.9
.1 —	1.5	.0 —	1.1	.2 —	1.2
.1 —	1.3	.0 —	1.4	.1 —	2.0
.1 —	2.7	112.9 —	2.2	.1 —	1.9
.1 —	1.9	.8 —	1.7	.0 —	1.1
.0 —	1.9	.8 —	1.0	.0 —	1.3
.0 —	1.8	.8 —	1.7	.0 —	1.9
.0 —	1.2	.8 —	1.2	.0 —	1.5
113.9 —	1.0	.7 —	1.5	110.9 —	1.6
.9 —	1.9	.6 —	1.2	.8 —	1.8
.9 —	1.3	.6 —	1.6	.7 —	1.4
.9 —	1.9	.6 —	2.2	109.8 —	1.3
.9 —	1.2	.6 —	1.4	.8 —	1.0
.9 —	1.2	.6 —	1.5	.3 —	2.2

B. STAT. 33. FREDERIKSSUND, ROSKILDE FJORD. AUTUMN 1915.

AVERAGE NUMBERS OF VERTEBRÆ IN THE DECADES AND FLUCTUATION OF SAME.

Population: **109.46** (Fluct. ± 0.49).

a	Fl.	a	Fl.	a	Fl.
115.9 —	± 1.7	110.6 —	± 2.0	109.5 —	± 1.1
114.1 —	2.3	.6 —	1.3	.5 —	1.1
113.4 —	1.4	.6 —	2.3	.5 —	1.6
112.7 —	1.2	.6 —	1.4	.5 —	1.8
.7 —	2.2	.6 —	1.3	.4 —	1.0
.6 —	1.5	.6 —	0.9	.4 —	1.4
.6 —	1.1	.6 —	1.2	.4 —	1.2
.5 —	1.5	.6 —	2.6	.3 —	1.5
.2 —	1.7	.5 —	2.0	.3 —	1.1
.1 —	1.1	.5 —	1.4	.2 —	1.0
.1 —	1.2	.5 —	1.8	.2 —	1.0
.0 —	0.7	.5 —	1.4	.2 —	2.0
.0 —	1.0	.5 —	1.5	.2 —	1.4
111.9 —	1.1	.4 —	1.0	.2 —	1.3
.6 —	1.7	.4 —	1.5	.0 —	1.0
.6 —	1.1	.4 —	1.8	.0 —	0.9
.4 —	0.9	.3 —	2.5	108.9 —	2.4
.4 —	1.8	.3 —	1.4	.9 —	1.4
.4 —	1.0	.3 —	1.0	.9 —	1.6
.4 —	1.5	.3 —	2.0	.9 —	2.6
.4 —	1.2	.2 —	1.3	.8 —	0.9
.3 —	1.7	.2 —	2.0	.7 —	1.5
.3 —	1.1	.2 —	1.0	.5 —	1.3
.3 —	1.3	.2 —	1.8	.5 —	1.1
.2 —	1.2	.1 —	1.6	.5 —	1.8
.2 —	1.7	.1 —	0.8	.4 —	1.6
.1 —	1.1	.1 —	1.2	.3 —	1.5
.1 —	1.1	.0 —	1.8	.3 —	1.5
.0 —	1.0	.0 —	1.5	.3 —	1.7
.0 —	1.0	.0 —	1.1	.2 —	1.3
.0 —	1.9	109.9 —	1.0	.0 —	2.6
.0 —	1.6	.9 —	0.8	107.9 —	2.1
.0 —	0.7	.9 —	1.3	.8 —	1.5
110.9 —	1.2	.8 —	1.2	.6 —	1.5
.9 —	1.1	.8 —	1.8	.6 —	2.0
.8 —	1.5	.8 —	1.7	.5 —	1.5
.8 —	1.1	.8 —	1.2	.5 —	1.3
.8 —	1.8	.7 —	1.3	.3 —	2.2
.7 —	1.4	.7 —	1.2	.1 —	3.4
.7 —	2.7	.7 —	1.3	106.6 —	1.6
.7 —	0.9	.6 —	1.8		
.6 —	2.0	.6 —	1.5		

C. STAT. 22. SNOGHØJ, LITTLE BELT. AUTUMN 1915.

AVERAGE NUMBERS OF VERTEBRÆ IN THE DECADES AND FLUCTUATION OF SAME.

Population: **117.44** (Fluct. ± 0.34).

a	Fl.	a	Fl.	a	Fl.
120.3 —	± 1.2	117.8 —	± 1.2	116.6 —	± 1.6
119.9 —	2.2	.7 —	2.2	.6 —	2.2
.8 —	1.6	.7 —	1.2	.6 —	1.1
.8 —	1.3	.7 —	0.7	.6 —	1.1
.7 —	1.4	.7 —	0.8	.6 —	1.6
.5 —	1.2	.7 —	0.9	.5 —	1.3
.5 —	1.2	.7 —	0.9	.5 —	1.3
.4 —	1.3	.7 —	0.9	.5 —	1.7
.3 —	1.0	.6 —	1.1	.5 —	0.8
.3 —	1.4	.6 —	0.9	.4 —	0.6
.2 —	1.7	.6 —	1.6	.4 —	2.1
.2 —	1.4	.6 —	1.1	.4 —	1.6
.2 —	0.8	.5 —	1.3	.4 —	1.0
.2 —	1.4	.5 —	1.1	.3 —	1.1
.1 —	1.1	.5 —	1.1	.3 —	0.9
.1 —	0.9	.5 —	1.1	.3 —	1.4
.0 —	1.1	.5 —	1.3	.3 —	1.2
118.9 —	1.2	.5 —	1.3	.3 —	1.3
.9 —	1.0	.4 —	0.9	.3 —	1.8
.9 —	1.2	.4 —	0.9	.3 —	1.5
.8 —	1.1	.4 —	1.4	.3 —	1.0
.8 —	1.0	.4 —	1.4	.3 —	1.5
.8 —	1.2	.3 —	2.0	.3 —	1.0
.6 —	1.3	.3 —	1.2	.2 —	1.2
.5 —	1.2	.3 —	1.7	.2 —	0.7
.5 —	0.9	.3 —	0.7	.1 —	1.1
.5 —	0.9	.3 —	1.2	.1 —	1.2
.5 —	1.4	.3 —	0.7	.1 —	1.9
.4 —	1.4	.3 —	1.4	.0 —	1.7
.4 —	1.8	.3 —	1.5	.0 —	0.7
.4 —	1.7	.2 —	1.0	.0 —	1.6
.4 —	0.9	.2 —	1.1	115.9 —	0.7
.3 —	1.3	.2 —	1.3	.9 —	1.6
.3 —	1.0	.2 —	0.8	.9 —	1.0
.3 —	1.0	.2 —	1.5	.9 —	1.8
.3 —	0.8	.1 —	1.4	.9 —	1.0
.3 —	1.5	.1 —	1.4	.9 —	1.3
.2 —	1.5	.1 —	1.4	.8 —	1.3
.2 —	1.2	.1 —	1.8	.8 —	1.1
.1 —	1.1	.1 —	1.5	.8 —	1.0
.1 —	1.5	.1 —	1.2	.8 —	0.9
.1 —	1.2	.0 —	1.1	.7 —	1.6
.0 —	1.1	116.9 —	1.0	.6 —	1.3
.0 —	1.1	.9 —	1.2	.6 —	2.0
.0 —	1.6	.8 —	1.0	.5 —	0.6
.0 —	0.9	.8 —	1.2	.4 —	1.4
.0 —	1.6	.8 —	1.4	.4 —	1.4
.0 —	1.1	.8 —	1.6	.3 —	1.4
117.9 —	1.1	.8 —	1.3	114.8 —	1.0
.9 —	1.1	.7 —	0.9	.7 —	1.2
.9 —	1.6	.7 —	0.9	.4 —	2.1
.8 —	1.5	.7 —	1.3	.4 —	1.3
.8 —	1.2	.6 —	0.8	.4 —	0.6
.8 —	1.2	.6 —	1.0	113.3 —	1.5

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XIV. Notes to the Charts Pl. I and II.

In Pl. I and II, the position of the stations is indicated by a circle with corresponding number, both in grey. The figure is the serial number of the station (or population) and refers to the List p. 351—359, where detailed information will be found.

The figures in colour are the average values found by population analysis for number of 1) rays in right pectoral fin (Pd) in blue, 2) vertebræ (Vert) in red, 3) hard rays in dorsal fin (D_2) in green, and 4) pigment spots on anterior soft-rayed part of dorsal fin (Pigm.D_1) in black.

Pl. I. The dotted line through Great Britain is intended to denote that *Zoarces* is only of common and regular occurrence east of the limit thus marked. From lack of sufficient data, however, this boundary must be emphatically stated as only approximate.

Pl. II. Stations 62. 63, 64, 65 and 66 were added after completion of the text. Their precise position will be seen from the list p. 359 and the results of the population analyses from here are noted in the tabular form on p. 367, 374, 381 and 388.

At the top of the chart are special diagrams on an enlarged scale showing Mariager Fjord, Kolding Fjord (both to the left) and Ise Fjord with Roskilde Fjord and Skjelskor Fjord (both to the right). Where no figures are marked in colour against a station on the main chart, the separate detail charts should be consulted.

Postscriptum.

Professor N. Knipovitch, of Petrograd, informs me in a letter dated $^{11}/_{6}$ 1917, that a description of *Zoarces elongatus*, with some information concerning the species, is to be found in a work by Peter Schmidt (in Russian) entitled "Pisces marium orientalium Imperii Rossici", which appeared in 1904. According to this writer, *Zoarces elongatus* greatly resembles *Z. viviparus*, but has a greater number of rays, the figures given being D: 88—90, IX—XII, 25—27; A: 96—98.

The local races of *Zoarces viviparus* L. Distribution of racial characters.

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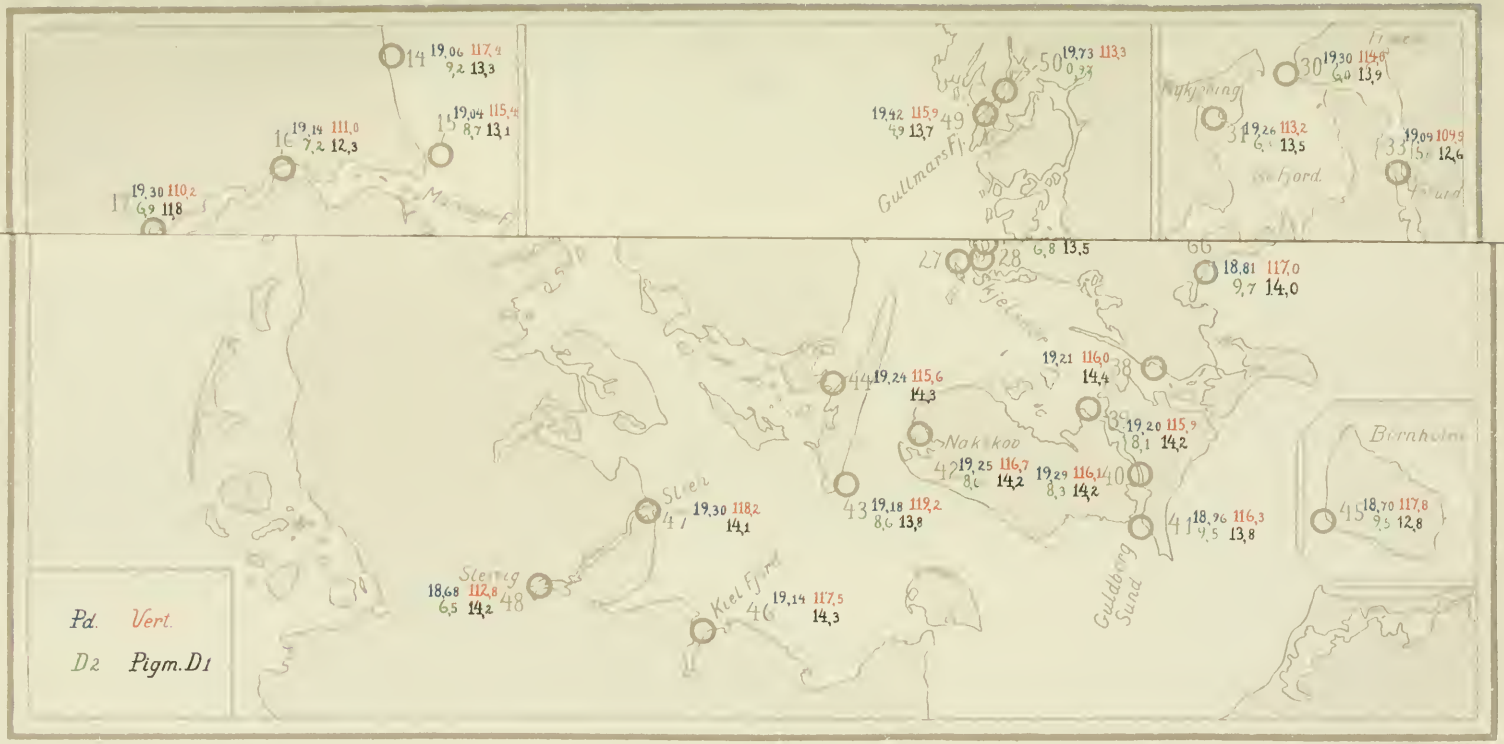
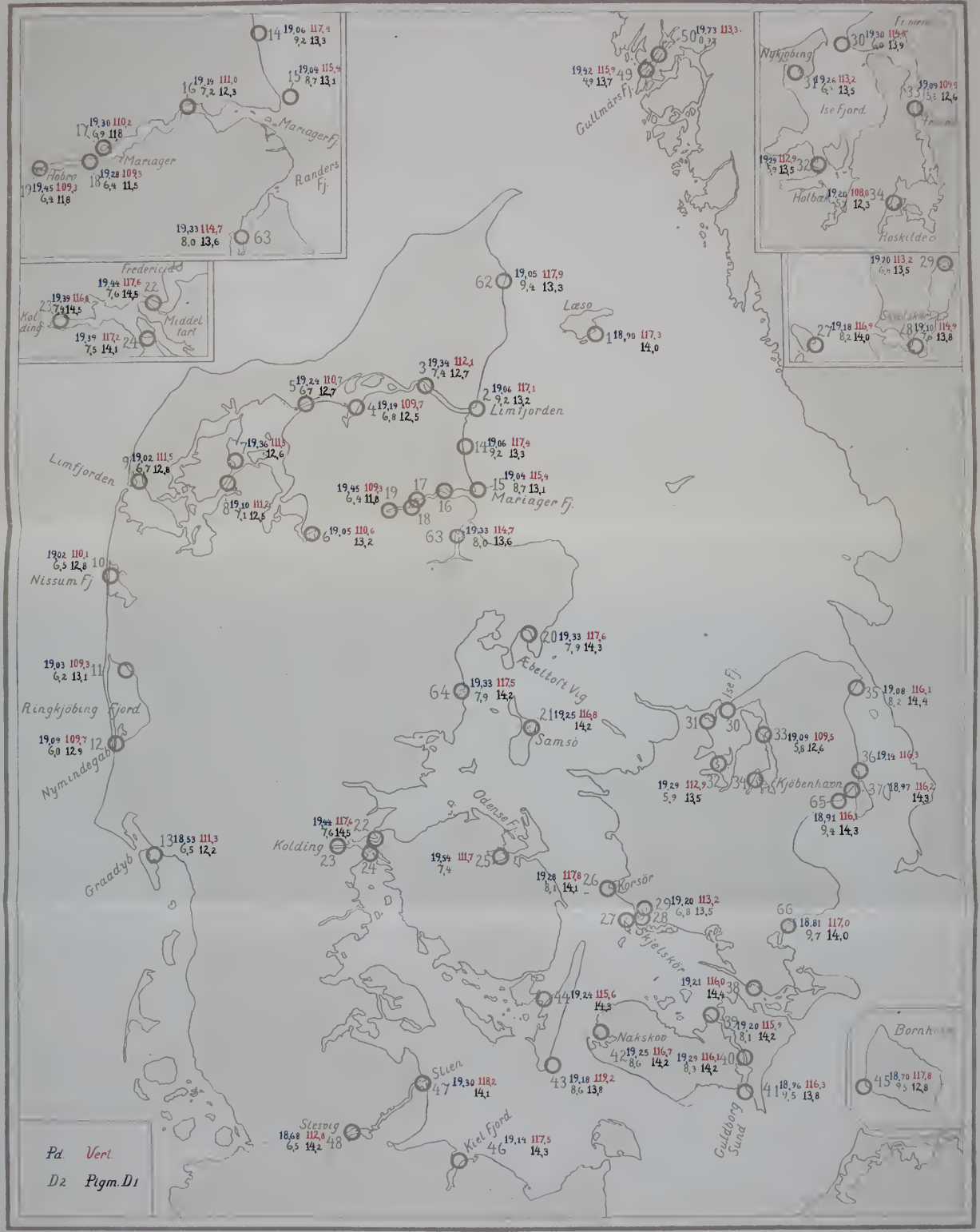
The local races of *Zoarces viviparus* L. Distribution of racial characters

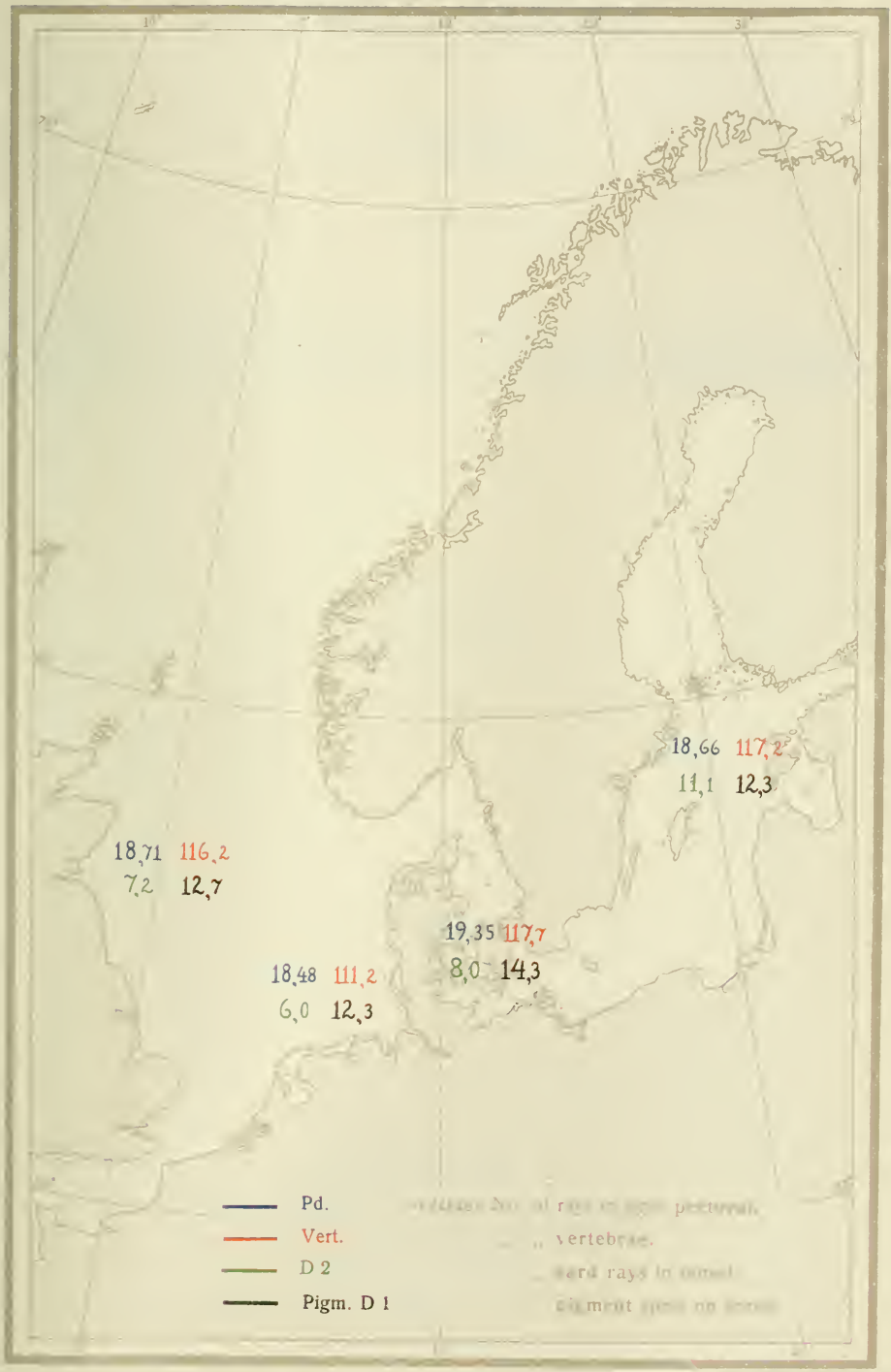
Approximate W. limit of regular occurrence known by dotted line.



The local races of *Zoarces viviparus* L. Distribution of racial characters.



The local races of *Zoarces viviparus* L. Distribution of racial characters.



The local races of *Zoarces viviparus* L.
Averages of racial characters in 6 areas.

COMPTES-RENDUS

DES TRAVAUX

DU

LABORATOIRE CARLSBERG

14ME VOLUME.

ÉDITION FRANÇAISE.

1917—1923

COPENHAGUE

EN COMMISSION CHEZ H. HAGERUP

IMPRIMERIE DE THIELE

1923

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JOHS. SCHMIDT:

RACIAL INVESTIGATIONS.

II. CONSTANCY INVESTIGATIONS CONTINUED.

BY

JOHS. SCHMIDT.

I. Introduction.

With investigations as to local races of fishes and other organisms, it must from the nature of the case be of great importance to ascertain whether the average values taken as characterising the races, (the "racial characters") remain constant in the same population from year to year. For it is obvious that all conclusions regarding the difference between two populations in respect of origin must be highly conditional, if it should be found that the qualities by which the populations are distinguished, themselves fluctuate greatly from year to year.

In the literature dealing with races of fish, we find here and there occasional investigations in this direction, as for instance in Heincke's great work on the races of the herring. Heincke was, he believed, able to show, by means of his population analyses from Slien in Slesvig. that the average racial characters were unchanged for the years 1878 and 1888 etc. (1898, I, p. XXVI and II, Tab. X—XV). Generally, however, too little attention has been devoted to this important question. Investigations have as a rule been restricted to the comparison, without further analysis, of populations from two or more waters, and the attempt to decide — by mathematical means — whether any difference could be discerned between the populations concerned.

Fishery biologists are as a rule chiefly interested in determining whether two or more waters have their own particular

stock of the species in question, or, in other words, whether the stocks are of different origin.

In approaching such questions, the customary method of procedure is to procure a sample from each of the two waters. With these samples as material, a great number of counts or measurements are made for the organs (vertebræ, fin rays, etc.) from which the racial characters are drawn. In other words, two population analyses are made. The numerical material thus procured is then subjected to mathematical treatment, according to one of the usual principles for variational-statistic investigations, the figures being inserted in the respective formulæ for determination of average, standard deviation, fluctuation, etc.

If then it is found that the application of the formulæ enables the investigator to determine the existence of a real difference between the average values for the two populations, it is usually considered justifiable to regard them as of different origin.

The question now is, whether we are warranted in drawing such conclusion; in other words, whether the numerical material obtained by ordinary population analyses is immediately suitable for such mathematical treatment, which makes no enquiry as to the nature of the material itself.

I shall in the following endeavour to answer this question by presenting the results of some of the investigations we have made in this direction. Later on, I hope to be able to give a more detailed account of the work.

The measurements given in Sections III and IV were carried out by myself, those in Section II by Hr. mag. sc. Vilh. Ege. The calculations were made by Frk. E. Fasting.

II. Analyses of a selected *Zoarces* population.

In a previous work, (1917) I have set forth the results of a great number of population analyses with *Zoarces* from Danish and adjacent waters. Among these, one group will be of particular interest in this connection, *viz.*, the series of analyses of one and the same population carried out annually for three successive years (St. 31, Nakkehage, Ise Fjord, near the mouth).

With regard to these so-called »constancy investigations« I stated (1917, p. 298): "Altogether, these repeated population analyses have revealed a considerable degree of constancy in the

different characters. In no case was any certain difference found between the average figures for the population samples taken at different times.

It will, however, as already mentioned, be necessary to continue the investigations for a considerable time yet, before a conclusive decision can be claimed."

The result then, of the population analyses in question was that no certain difference could be discerned between the three samples taken at yearly intervals from one and the same population. It might, however, be imagined, that a difference did in reality exist between year-classes of *Zoarces*, but was concealed by the fact that each of the population samples examined consisted of several year-classes mixed together. This last is, as a matter of fact, the case, the usual samples being generally found to contain at least three different year-classes, *viz.* two-, three-, and four-year-old fish, indiscriminately mingled.

It was consequently desirable to carry out a closer analysis, by taking the different year-classes separately. And I am now able to communicate the results of such investigation, made with material from the innermost part of Roskilde Fjord (St. 34, see Fig. p. 6. The population of this locality is distinguished by having the lowest average number of vertebræ hitherto found, *viz.* abt. 108, and it is also in other ways particularly suited for our purpose, e. g. in the fact that the youngest year-classes are here unusually easy to obtain.

The method employed was as follows: Some large population samples were procured from St. 34, and the age of each single specimen determined by means of the scale rings, which in *Zoarces* are as a rule extremely distinct. This was done prior to counting the vertebræ, fin rays, and pigment spots. Wherever the slightest doubt existed as to age, the specimen in question was discarded. The wastage thus caused amounted in all to some 7 %, which is, however, of but little importance where a sufficiently extensive material is available.

In the accompanying table will be found the results of these counts of hard rays in dorsal fin (D_2) and rays in right pectoral fin, with the corresponding average figures, etc. The figures noted in parentheses refer to the left pectoral fin (Ps.).

As regards the hard rays (D_2) it will be seen that there is a considerable difference between the year-classes 1915 and

Table I. — *Zoarces*, Stat. 34, Roskilde Fjord, Langholm[1]).

Hard rays in dorsal fin (D_2).			Rays in pectoral fins (Pd og Ps).					
No. of rays	Year-class 1915	Year-class 1916	No. of rays	Year-class 1915		Year-class 1916		
				Pd	Ps	Pd	Ps	
10	4	2	21		3	3	3	
9	19	1	20	63	79	129	142	
8	88	18	19	305	295	162	143	
7	126	44	18	66	71	10	12	
6	93	107	17	4	4			
5	64	74						
4	11	29						
3		2						
n	405	277	n	438	(452)	304	(300)	
a	**6.714**	**5.830**	a	**18.975**	**(19.013)**	**19.411**	**(19.453)**	
σ	± 1.234	± 1.139	σ	± 0.577	(± 0.629)	± 0.573	(± 0.592)	
P. E. A.	± 0.041	± 0.046	P. E. A.	± 0.019	(± 0.020)	± 0.022	(± 0.023)	
Fl.	± 0.205	± 0.230	Fl.	± 0.095	(± 0.100)	± 0.110	(± 0.115)	

[1]) The following formulæ were employed:

σ ("standard deviation") $= \pm \sqrt{\frac{\Sigma a p^2}{n-1} - b^2}$

P.E.A. ("probable error of average") $= \pm \frac{\sigma}{\sqrt{n}} \cdot 0.67449$

P.F.A. ("probable fluctuation of average") $= \pm \frac{\sigma}{\sqrt{n}} \cdot 0.67449 \cdot 5$

1916 (born in Jan.—Feb. 1915 and Jan.—Feb. 1916 respectively), the average values being 6.714 and 5.830 respectively, and as the number of specimens was in each case large, (405 and 227) the values are determined with considerable certainty. Arithmetical treatment then shows that the true average of the year-class 1915 can with a probability of 13—1400 to 1 be taken as lying between 6.509 and 6.919, and for the 1916 year-class between 5.600 and 6.060.

Turning then to the pectoral fins, we find that the 1915 year-class has 18.975 rays in average, whereas the value for 1916 fish is essentially higher, *viz.*, 19.411 (for the left pectoral fin, the figures are 19.013 and 19.453 respectively). The true average then lies, with a probability of 13—1400 to 1, between 18.880 and 19.070 for the 1915 year-class, and between 19.301 and 19.521 for that of 1916.

There can thus be no doubt that the two year-classes really differ in respect of the characters investigated, the difference being, moreover, in part quite as great as that found to exist between many pairs of populations which are geographically very wide apart. I may here refer the reader to my previously mentioned work, where the results of 61 population analyses are noted in the two charts (1917, Pl. I—II).

We must now ask whether it can be considered certain that the two year-classes 1915 and 1916, as distinguished by our age determinations, really belong to the same population. Both were taken at the same locality, *viz.* St. 34 in the innermost part of Roskilde Fjord, but it might still be imagined that the one year-class could, for instance, have originated from another water, and have migrated to St. 34[1]). This is, however, improbable on the whole, from the slight power of migration evinced by our species, which renders it far better suited to decide questions of this nature than most other species of fish, more capable of active or passive migration.

Furthermore, in the particular case with which we are here concerned, there are certain local conditions which practically

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[1]) A. C. Johansen has, in one of his works on the biology of the plaice, (1910, p. 123) carried out several population analyses, where the 0 and 1 groups were examined separately for number of anal rays. The writer uses his material to show that an immigration from alien populations does take place. It is not shown whether there was a difference anywhere between two year-classes belonging to the same population.

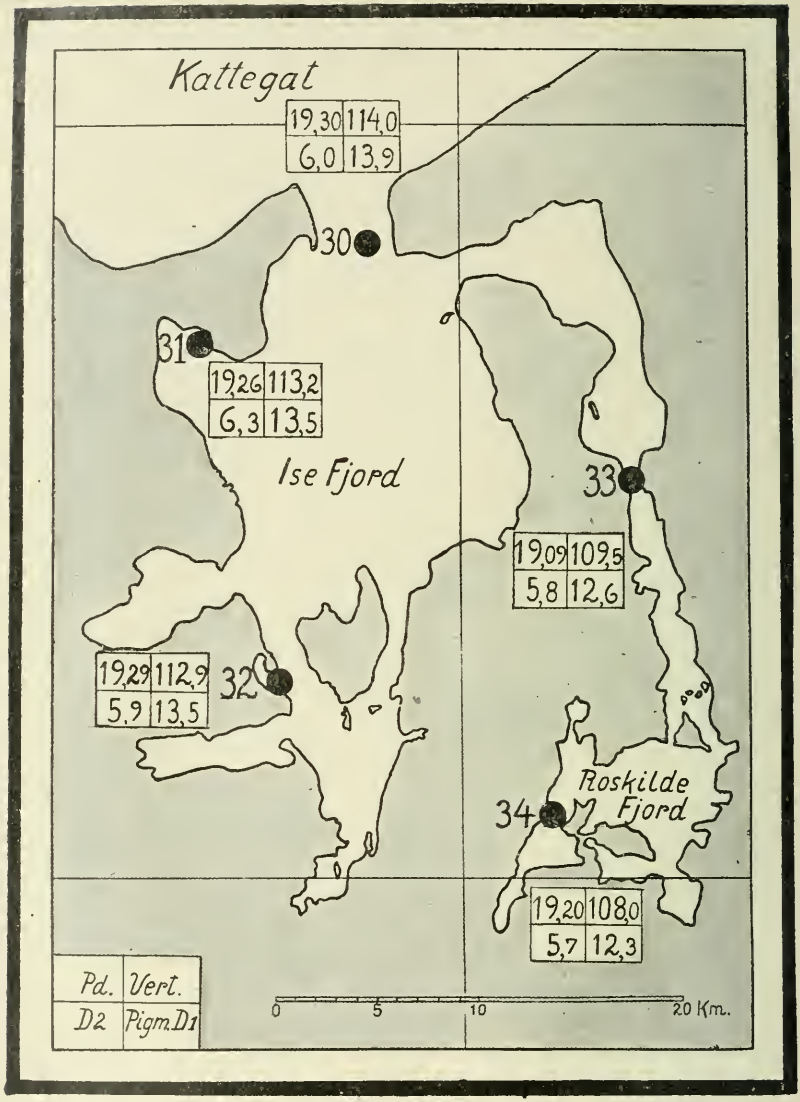

Chart of the Ise Fjord with Roskilde Fjord.
The black spots with serial numbers (30—34) indicate the localities (the "Stations") where ordinary population analyses of *Zoarces* were made. The figures in the squares give the mean values for the 4 characters investigated. (From Johs. Schmidt, 1917, Pl. II).

render it out of the question that the difference found between year-classes 1915 and 1916 should be due to immigration from alien populations.

The accompanying figure gives a sketch of Roskilde Fjord and Ise Fjord, of which the Roskilde Fjord is a lateral branch. The four stations where population analyses were made are noted on the figure, as also the average values found for the four characters: number of pectoral rays, vertebræ, hard rays and pigment spots. It will be seen from the figure that St. 34 is situated at the base of the funnel-shaped expansion of Roskilde Fjord, and that any possible immigration of individuals with essentially different qualities could only take place from the north, through the long narrow channel forming the outermost part of the fjord where it runs out into the Ise Fjord. Here, however, we have several investigation stations, and are thereby enabled to estimate whether any immigration could have taken place from the north. That such has not been the case appears with a high degree of probability from the fact that both year-classes 1915 and 1916 from St. 34 have the same, very low average number of vertebræ, abt. 108[1]), as found by the very first population analyses for this station, and in which it differed from all other localities investigated.

The result of our investigations then, must be said to be that it has been shown, with as high a degree of certainty as is anywhere attainable in investigations of this character, that two year-classes of one and the same population can differ in respect of two of the characters investigated. These are, the number of pectoral rays and number of hard rays in dorsal fin. We have, however, in no case been able to find any certain difference between year-classes in respect of the other two characters examined, the vertebræ and pigment spots[2]).

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[1]) Year-class 1915 had in average 108.224 (Fluct. ± 0.320) vertebræ and 12.337 (Fluct. ± 0.330) pigments spots; year-class 1916 108.077 (Fluct. ± 0.385) and 12.454 (Fluct. ± 0.395) respectively).

[2]) In the constancy investigations referred to in my previous work (1917, p. 296—297) from St. 31 the mouth of the Ise Fjord, where the population samples were not sorted out into year-classes, we did not succeed in demonstrating any certain difference between samples taken from the population during the three years 1914, 1915 and 1916. The average values for hard rays and pectoral rays, however, showed somewhat less agreement than did the vertebræ and the pigments spots. The average values found for the three years were as follows (figures in parentheses denote fluctuation). Hard rays: 6.49 (± 0.39), 6.32 (± 0.34), 6.12 (± 0.31), Pectoral rays 19.43 (± 0.15), 19.26 (± 0.13), 19.26 (± 0.14).

Dr. A. C. Johansen, of Copenhagen, has with regard to this in a

III. Comparison of females and their offspring within two *Zoarces* populations.

Besides being highly localised, *Zoarces* is also in other ways particularly adapted to the elucidation of questions such as that with which we are here concerned. The fact of its being viviparous enables us to compare qualities in the mothers with those in their young, or rather, in their embryos. Further details regarding such investigations I have already described in my oft-quoted work (1917, p. 316) to which the reader may refer.

In the previous section, it was seen that we had succeeded in demonstrating the existence of a difference between two year-classes of the same population. It was pointed out, however, that any such demonstration demands proof that no intermixture of alien elements from other populations has taken place. Such proof can of course only be one of probability, and even this may often be very difficult to procure. We have, however, in *Zoarces* the possibility of obtaining direct proof, by comparing the gravid fermales in a population sample with the embryos they contain.

In the accompanying table II will be found the results of such an investigation, from St. 40 in Guldborgsund, Nykjøbing (see 1917, pl. II, for position of the station in question).

The character investigated was the number of rays in right pectoral fin (Pd). In the second column will be found the number of rays in the mothers, while the two last columns give the figures found for their offspring. The figures in columns III and IV refer to samples procured by taking out 3 and 1 embryos respectively from each mother[1]).

---

letter of $^3/_9$ 1917, stated as follows: "True, no certain difference has been shown to exist in any case between the average figures, but it is nevertheless most probable that a difference does exist, as for instance at St. 31, Ise Fjord, between the hard ray values for 1914 and 1916, and number of rays in right pectoral fin for 1914 as against 1915 and 1916."

The investigations described in the present paper, from which, as we have seen, it was found that just these particular values, the number of hard rays and pectoral rays, might differ from generation to generation, render Dr. Johansen's supposition highly probable. If any difference really exists, it would also no doubt have been more distinctly apparent had the population samples in question been sorted out into the various year-classes of which they were composed.

[1]) Such samples we term "representative offspring samples", as they represent the population of the place, or more correctly, a single year-class of the same, which we are thus enabled to investigate without having first to make

Table II. — *Zoarces*, Stat. 40, Nykjøbing, Falster.

Rays in right pectoral fin (Pd).

No. of rays	Gravid ♀♀	Embryos (3 from each ♀)	Embryos (1 from each ♀)
21	1		
20	39	47	14
19	94	255	84
18	7	97	36
17		1	
n	141	400	134
a	**19.241**	**18.870**	**18.836**
σ	± 0.547	± 0.597	± 0.601
P. E. A.	± 0.031	± 0.020	± 0.035
Fl.	± 0.155	± 0.100	± 0.175

It will be seen from the figures shown, that the mothers have here, on the average, a greater number of pectoral rays than the offspring, viz. 19.241 as against 18.870. The usual arithmetical treatment of the material then gives the result that the true average lies, with a probability of 13—1400 to 1, in the case of the mothers between 19.086 and 19.396, and in that of the offspring between 18.770 and 18.970.

A further example will be found in Table III with material from Roskilde Fjord (see chart-sketch p. 6). Here also we are dealing with number of rays in right pectoral fin. The average figures for the mothers are here seen to be lower than those for the offspring, *viz.* 19.070 as against 19.343. With the same degree of probability as before (13—1400 to 1) the true

lengthy age determinations. Besides samples with 1 and 3 embryos, others were also taken with 2 and 4 from each mother. We are as a rule not able to examine them all, but they are preserved for reference in case of need, and this may the more easily be done as they take up no room to speak of in comparison with the other population samples consisting of adult specimens.

It will be noticed that the number of specimens in column 4 of Table II is 7 less than that of the mothers from which they were taken. This is due to the fact that 7 embryos had to be discarded owing to the staining having failed, or on account of malformation, or damage incurred during preparation. Something similar applies in the case of column 3.

Table III. — *Zoarces,* Stat. 33. Frederikssund (Roskilde Fjord).

Rays in right pectoral fin (Pd).

No of rays	Gravid ♀♀	Embryos (10 from each ♀)	Embryos (1 from each ♀)
21	3	24	2
20	22	488	50
19	85	671	67
18	17	97	9
17	1		
n	128	1280	128
a	**19.070**	**19.343**	**19.351**
σ	± 0.654	± 0.643	± 0.636
P. E. A.	± 0.039	± 0.012	± 0.038
Fl.	± 0.195	± 0.060	± 0.190

average for the mothers will lie between 18.875 and 19.265, in the young between 19.283 and 19.403, and they thus appear, as in the previous case, to differ, albeit the difference is but slight.

By this means of investigation, we have only succeeded in demonstrating a difference between mother and offspring in respect of the one character, number of pectoral rays, not in the case of vertebræ or pigment spots, despite the fact that a great number of investigations have already been made. As regards the hard rays in dorsal fin, we were obliged to disregard this character in the embryos, as it was not always possible to distinguish with certainty the limitations of the hard-rayed part of the fin. In the case of the three remaining characters, there is no such restriction; the values can be determined with certainty in the embryos, where the full complement is already present at an early stage.

One other point should, for the sake of completeness, also be noted. As the offspring samples consist of both males and females, it would not be justifiable to compare them with the mothers, unless it had previously been demonstrated that no difference existed between the two sexes in respect of the characters investigated. This however, I have already shown to be the case (1917, p. 289).

The results of the investigations with *Zoarces* described in Sections II and III may then be summed up as follows: It has been shown that two year-classes of the same population may differ in two of the four characters investigated, *viz.* the number of pectoral rays and number of hard rays. For the other two characters, no such difference could be discerned.

IV. Experimental investigations with *Lebistes reticulatus* (Peters) Regan.

Lebistes reticulatus is a small fresh-water fish from the West Indies and South America, belonging to the Cyprinodonts, which has of late years been largely introduced into indoor aquaria. Like *Zoarces*, it is viviparous, and the females will, under suitable conditions of temperature etc., bring forth, at intervals of about four weeks, a considerable number of young, not infrequently about fifty at a time.

I have during the last few years carried out a considerable number of experimental investigations with this species, and one series deserves to be mentioned in connection with the *Zoarces* investigations dealt with in the foregoing. In the matter of furnishing exact proof, we are in some respects even better situated with a species like this than with *Zoarces*, since we are here able to keep the specimens under control from the moment of birth, and isolate them in pairs, which again permits us to compare the offspring, not only with the mother, as in *Zoarces*, but with both parents.

The character which I have here selected is the number of dorsal fin rays. The value for this is stated by Tate Regan, (1913, p. 1008) one of the best authorities on these fish, as 7—8. In the course of the many hundreds of counts which I have made, the range of variation has not unnaturally been somewhat extended, *viz.* to between 5 and 8. The very great majority of specimens, however, have 7 rays in the dorsal fin.

The principle on which these investigations were based was to vary the external conditions for the same pair of parents from one period of gravidity to the other, and then determine the number of dorsal fin rays in the various broods of offspring.

The external factor to be considered in our investigations is

the temperature. At the time when the investigations were carried out, we had no means of maintaining a constant temperature in our aquaria. And in stating that the specimens were kept at a "low", "medium" or "high" temperature, we thereby understand "medium" as being abt. 6^0 above "low" and abt. 3^0 below "high". Generally speaking, "low" temperature was equivalent to abt. 19^0, fluctuating between abt. 17^0 and abt. 23^0. As far as possible, the aquaria were kept uniform in other respects, such as water level, plant contents, quantity of food. etc. The fish were fed with dried Daphnia.

The results of these investigations can be seen from Tables IV—VIII, each of which shows the number of rays in several broods from the same pair of parents. The date of birth of the young is noted in each case, as also whether developed at low, medium or high temperature.

The transference of the parents from "high" to "low" temperature or the reverse was made on the day when the female had brought forth its young. The latter are born with their full complement of rays, so that the treatment of the young after birth is of no importance. We invariably placed them at a high temperature, to accelerate growth.

On going through the tables, it is distinctly evident that the different broods do exhibit a difference in the number of dorsal fin rays, and it is a further seen, that the average number of rays was greater where the young had been developed at high temperature than where their development took place at low.

Generally speaking, the difference between the "cold-water" and "warm-water" broods was not great. In some cases, however, it was comparatively speaking very considerable, as for instance the May and June broods in Table IV. The average values here are abt. 7.6 and 6.7 respectively, the difference being thus no less than 0.9. Still more forcible proof however, is afforded by the fact that the influence of temperature tended in the same direction in all the 15 experiments. There is not a single exception anywhere, though the effect is not always discernible to the same degree.

That it really is the temperature which has directly or indirectly affected the specimens, would thus seem beyond doubt. I may also refer to the case in Table IV, where it will be seen

Table IV.

No. of rays in dorsal fin (D) for offspring of ♂ D_7 × ♀ D_7.

No of rays	High temperature Born 12. March	Medium temperature Born 13. April	Low temperature Born 1. June	Low temperature Born 25. July	High temperature Born 25. Sept.
8	9			1	4
7	6	20	25	33	16
6			13	4	
n	15	20	38	38	20
a	**7.600**	**7.000**	**6.658**	**6.921**	**7.200**
σ	± 0.532		± 0.493	± 0.390	± 0.414
P. E. A.	± 0.093		± 0.054	± 0.043	± 0.062
Fl.	± 0.465		± 0.270	± 0.215	± 0.310

Table V.

No. of rays in dorsal fin (D) for offspring of ♂ D_8 × ♀ D_8.

No of rays	Medium temperature Born 12. April	Low temperature Born 29. May
8	15	
7	29	27
6		5
n	44	32
a	**7.341**	**6.844**
σ	± 0.483	± 0.399
P. E. A.	± 0.049	± 0.048
Fl.	± 0.245	± 0.240

Table VI.

No. of rays in dorsal fin (D) for offspring of ♂ D_8 × ♀ D_7.

No of rays	Medium temperature Born 23. May	Low temperature Born 14. July
8	8	
7	49	31
6		6
n	57	37
a	**7.140**	**6.838**
σ	± 0.351	± 0.399
P. E. A.	± 0.031	± 0.044
Fl.	± 0.155	± 0.220

Table VII.

No. of rays in dorsal fin (D) for offspring of ♂ D_6 × ♀ D_6.

No of rays	Medium temperature Born 1. April	Medium temperature Born 27. May	Low temperature Born 13. July
8	1	1	
7	11	31	15
6	3	6	21
n	15	38	36
a	**6.867**	**6.868**	**6.417**
σ	± 0.565	± 0.439	± 0.505
P. E. A.	± 0.098	± 0.048	± 0.057
Fl.	± 0.490	± 0.240	± 0.285

Table VIII.

No. of rays in dorsal fin (D) for offspring of ♂ D_6 × ♀ D_6.

No of rays	Medium temperature Born 7. May	Low temperature Born 19. June	Low temperature Born 14.-15. August
8			
7	11	17	35
6		11	18
5		2	
n	11	30	53
a	**7.000**	**6.500**	**6.660**
σ		± 0.636	± 0.487
P. E. A.		± 0.078	± 0.045
Fl.		± 0.390	± 0.225

that we succeeded in getting no less than 5 broods to develop at different temperatures, with the result that a fairly distinct trend is discernible in the average number of rays, agreeing with the course of the temperature. I do not, however, propose to discuss this question further here, since we are for the present only concerned with ascertaining how far any difference at all could be shown to exist between different batches of young from the same parents.

V. What conclusions can be drawn from the present investigations?

We have in the foregoing endeavoured to see whether it was possible to demonstrate the existence, within the same population etc., of differences appearing from generation to generation, or from one brood to another, in the average qualities utilised as racial or specific characters in fish. As we have seen, the investigations in question, which were carried out by means of three different methods, gave positive results for several characters, differences of the nature in question being actually found to exist.

The natural consequence must then be a warning to observe the greatest caution in drawing conclusions as to difference of origin on the basis of ordinary population analyses. This is perhaps most distinctly evident from the figures shown in Table IV under dates of 12 March and 1 June. The average values here are 7.6 and 6.7. On seeking to determine, by purely mathematical means, whether two populations exhibiting such numerical proportions were of different origin, the answer would presumably be in the affirmative. Nevertheless, the facts here show, that these figures are derived from an investigation of the progeny produced by the union of a single pair of individuals.

In population analyses, therefore, it will be necessary, before proceeding to mathematical treatment of the material, to subject the same to the keenest possible biological analysis, if the results are to be of any value in drawing conclusions as to difference of origin between the populations concerned.

In my previous work on *Zoarces* I mentioned (1917, p. 342) that the cause of racial differences in fish should probably be sought first of all in the dissimilar mixing proportions of the genotypes, and only secondarily in the environment.

The present paper furnishes proof of the importance of environment, but as regards the permanence of the types, I reserve the elucidation of this for a subsequent occasion.

VI. Summary.

The present work is concerned with the constancy of such characters as are employed in distinguishing between races of fish.

The material employed in the investigations consisted of numerous populations of *Zoarces viviparus* L. from Danish waters. The following characters were examined: number of vertebræ and pectoral rays, and number of hard rays and pigment spots in the dorsal fin (Sections II—III).

Furthermore, experimental investigations were carried out in aquaria with the West Indian Cyprinodont, *Lebistes reticulatus* (Peters) Regan, for the purpose of elucidating the question as to constancy of the average number of dorsal rays (Section IV).

The investigations were carried out in the following manner. To begin with, ordinary population samples of *Zoarces* were resolved, by age determination of each individual specimen, into the different year-classes of which they were composed. These year-classes (generations) could then be analysed separately, and their qualities compared (Section II).

In the second place, all gravid females in several *Zoarces* populations were analysed, as also samples of the embryos contained. It was thus possible to compare directly the qualities in different year-classes of fish (Section III).

Thirdly, with material of *Lebistes reticulatus*, various broods of young derived from the same parents, but developed under different external conditions, were investigated, whereby the importance of environment could be directly put to the test. (Section III).

Many of the experiments with *Zoarces* gave negative results, no difference being discernible between the average qualities of the different year-classes. This applies, for instance, to all investigations hitherto made with regard to number of vertebræ and of pigment spots. In several cases, however, we succeeded in proving that there can be a considerable difference within one and the same population from one year-class to another, both as regards number of hard rays in dorsal fin, and number of rays in pectoral fin (Section II—III).

The experiments with *Lebistes reticulatus* led to the result that the temperature exerted a certain influence upon the average

number of rays in the dorsal fin in various broods from the same parents (Section IV).

It follows from these investigations, that population analyses which, — as is usually the case — are carried out with samples consisting of several year-classes, are not suited to the purpose of dealing with the question as to constancy of racial characters, as possible differences between the separate year-classes may be obscured by the intermingling of one year-class with another. It is further seen, that the greatest caution should be observed in drawing conclusions as to difference of origin between two populations on the basis of ordinary population analyses. Altogether, the results here set forth warn us that all such material should be subjected to the closest biological control before we can proceed to deal with it by the customary mathematical processes, in which no regard is taken to the nature of the material itself.

Carlsberg Laboratorium, 5. October 1917.

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JOHS. SCHMIDT:

INVESTIGATIONS ON HOPS

(*Humulus Lupulus*, L.)

XI. CAN DIFFERENT CLONES BE CHARACTERISED BY THE NUMBER OF MARGINAL TEETH IN THE LEAVES?

BY

JOHS. SCHMIDT.

I. Introduction.

In all biological work with quantitative characters, whether systematic or genetic studies, one question repeatedly asserts itself. Are the numerical differences which we find in our plants and animals to be regarded as indicative of genotypic differences, or are they merely of a phænotypical nature; i. e. produced by the external conditions under which the organisms in question have developed?

Such questions have been dealt with in the first paper of my "Racial Investigations"[1]), dealing with local races of the Viviparous Blenny (*Zoarces viviparus* L.). I pointed out there the necessity of experimental work if we are to get beyond mere supposition. And experimental researches with this and other species of fish have accordingly been commenced, some being already brought to a conclusion[2]).

In other ways, also, impracticable with fishes, which present various difficulties for experimental investigation, we have at Carlsberg Laboratory endeavoured to throw light upon questions of this nature. There are for instance our experiments with hop

[1]) Johs. Schmidt: Racial Investigations No. 1. *Zoarces viviparus* L. and local races of the same. (Comptes-rendus des travaux du Laboratoire de Carlsberg, 13. 277—397 (1917)).

[2]) *Id.*: Racial Investigations No. 2. Constancy Investigations continued. *Ibid.* 14. 1—19 (1917).

plants (*Humulus lupulus* L.), of which I shall here give a brief report.

The hop is a perennial plant, easily capable of vegetative propagation It is thus possible to study and compare qualities in one and the same individual — the same genotype — during different years. By dividing the individual plant, we are able to test simultaneously the effect of different external conditions upon the same genotype. In principle then, the hop plant is excellently suited to experiments such as those proposed.

Fig. 1. Leaf of Hop (*Humulus lupulus* L.). Showing manner of counting the leaf-teeth.
All the teeth of the terminal lobe, i. e. those between the two upper horizontal dotted lines in the figure, are included. The leaf shown has 26 teeth.
The line "L" indicates the length of the leaf, measured in classifying the leaves according to size (cf. p. 3).

The next thing to do was to find a character in the hop plant suitable for the purposes of investigation.

After various preliminary studies, I fixed upon the number of points on the margin of the middle lobe of the leaf.

We also endeavoured to find suitable figures from the flora portions of the plant, but relinquished these as apparently lessl adapted for our purpose. Moreover, the vegetative leaves were preferable, as the floral ones are quite different in male and

female, and are therefore useless for comparison of the seedlings (the offspring of crossing) with both parents.

The teeth on the terminal lobes of the leaves are well separated from those of the lateral lobes, the basal portion of the former having no indentation of the margin at all.

A glance at the accompanying figure (1) will explain better than words the manner in which the counting of the points was carried out.

Leaves on axes of the first order[1]) are larger than those of the secondary axes, and it was at once noted that the former have more points than the latter. We therefore decided to keep to leaves of secondary axes, which of course are far more numerous, and for that reason also better suited to purposes of investigation than leaves of the primary axes.

In order to render the material as uniform as possible, we worked throughout with leaves from a certain height on the plant, *viz.* from $1^1/_2$ to $3^3/_4$ metres above ground, i. e. on a range of $2^1/_4$ metres; within this range, all leaves of the secondary axes were picked off for investigation.

The material was collected in late summer, and was as a rule examined while still fresh. At times also material preserved in spirits was employed.

When counting the teeth of a leaf, the leaf itself was classified according to size (length). The length was measured, as indicated in fig. 1, from the juncture between stalk and leaf to the point of the leaf itself (of the median lobe). Seven groups were established for length: < 2, 2—4, 4—6, 6—8, 8—10, 10—12, > 12 cm. The leaves of each lateral shoot were separately examined, and note was taken of the position of the lateral shoots (axes of the second order) on the main axis (height above ground).

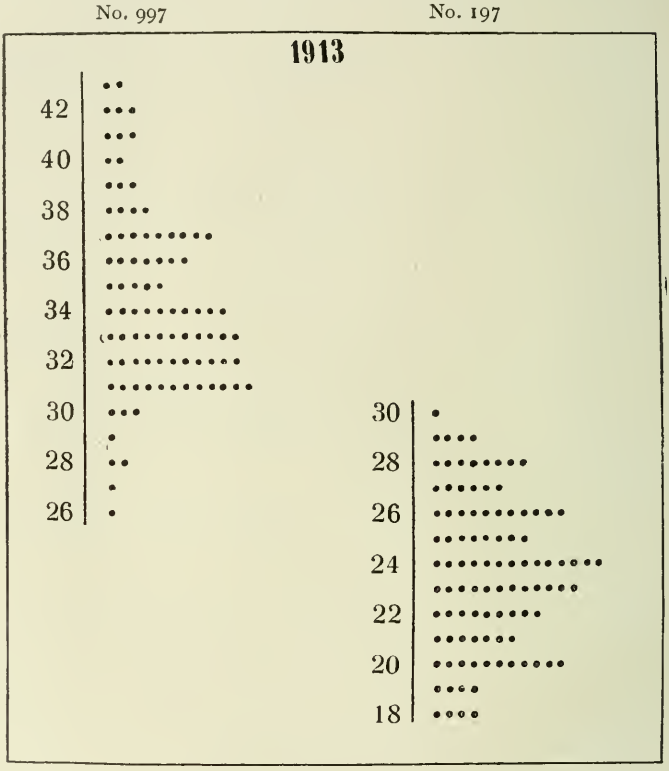
The extensive labour of counting and calculation was carried out by Frk. E. Fasting, to whom I beg to express my best thanks for the care devoted to the task. I am greatly indebted also to Dr. Ö. Winge, who supervised the work.

---

[1]) It should be noted that there is here only question of the axes above ground. These, which die off each year, are lateral shoots from the persistent underground shoots.

II. Preliminary Investigation.

A preliminary investigation was carried out in 1913 with two female plants, an American "Oregon Cluster" No. 997, and a Danish "Grøn" No. 197. The former belongs to the same clone[1])



No. 997		No. 197	
n	= 90	n	= 99
a	= **34.433**	a	= **23.667**
σ	= ± 3.718	σ	= ± 3.014
P. E. A.	= ± 0.264	P. E. A.	= ± 0.204
Fl.	= ± 1.320	Fl.	= ± 1.020

Fig. 2. No. of leaf-teeth in 1913 in 2 plants belonging to the clones "Oregon Cluster 60" (left) and "Grøn 197" (right).

as "Oregon Cluster 60", which was previously used for investigations on the inheritance of aroma in hops (Investigations on Hops (*Humulus lupulus* L.) No. V: "On The Aroma of Hops";

[1]) The term "Clone" is further explained in the above-quoted work on Aroma in Hops (p. 152—53, foot-note). A hop clone comprises the hop plants which can be produced by vegetative propagation of one and the same seedling. A clone plant is any single plant belonging to the clone.

Comptes-rendus des travaux du Laboratoire de Carlsberg **11**, 149—163, 1915). "Grøn No. 197" was from the hop growing districts of Funen.

The results of the preliminary investigation will be seen from the accompanying graph fig. 2. The figures on the vertical lines indicate the number of teeth on the median lobe of the leaves, the black spots denoting the number of leaves.

It will be seen from the graph that there was a considerable difference between the two plants, the American having a markedly higher average number of leaf-teeth than the Danish plant. The average number for "Oregon Cluster" No. 997 was abt. 34.43, and that for "Grøn" No. 197 only abt. 23.67.

The question then was, whether this difference was independent of the external conditions, or if perhaps it was only a result of difference in external conditions. In order to decide this point, various investigations were made, which will now be described in the following section.

III. The same clone through three different years.

For investigation we selected a plant from Worcestershire, in England, "Mathon No. 760" which we had cultivated for several years, and of which we had a large material of plants raised by vegetative propagation (clone plants) in our experimental nursery.

The object of the investigation was to ascertain whether the number of leaf teeth in this clone remained constant from year to year. The plants in question grow in two different rows in the nursery, and their bines are, according to the Kentish system, trained up strings of cocoanut fibre, stretched between two horizontal lines of wire. Each plant has three strings allotted to it, and two bines are trained up each string.

The three years during which the investigations were carried out were 1915, 1916 and 1917; in the case of some plants, only the two last years.

The results of the investigation will be found in concise form in Table I, showing the number of leaves examined (n), the average number of leaf teeth (a), standard deviation (σ), probable error of average (P.E.A.) and probable fluctuation of same (Fl.). The last value but one is calculated according to the formula:

$$\frac{\sigma}{\sqrt{n}} \cdot 0.67449, \text{ the last according to } \frac{\sigma}{\sqrt{n}} \cdot 0.67449 \cdot 5 .$$

In addition, the results of investigation of 2 plants (Nos. 1176 and 1185) are graphically shown in figs. 3—4, with 3 graphs for each plant, the first for the year 1915, the second for 1916, and the third for 1917. As in the case of the first graph p. 4, the figures along the vertical lines indicate the number of leaf-teeth, while each dot denotes one leaf investigated. In order to facilitate comparison between the three years, a horizontal line has been drawn off the number 26 for leaf-teeth.

It will at once be seen from the graphs that the number of leaf-teeth differed for the three years. The average number was lowest in 1915, higher in 1916, and at its maximum in 1917, the figures being, for No. 1176, abt. 22.46, 26.05 and 28.38 respectively; for the other plant, (No. 1185) abt. 22.9, 26.06 and 29.07.

The figures for the other plants noted in the table, all of which belong to the same clone, the same genotype, as the one noted in the graphs, show that they are almost without exception the same as this; the average no. of leaves is lowest in the first year, rising in the next or the next two.

We have seen, then, that a difference is discernible in the numerical values for the same plant in different years, and we have also observed a certain definite course in the variation. The question now is, what can be the cause of this difference, and of the regular increase in the average figures.

Nothing was done to vary the quantity or quality of the nutritive substances conducted to the plants through the roots during the years of experiment; it is thus natural to consider whether climatic conditions might not be the cause, the more so since the summer of 1916 was unusually cold and that of 1917 exceptionally hot.

On closer examination of the table, however, it is evident that climatic conditions cannot be, at any rate wholly, responsible. We find in 1916 a plant, No. 1148, which in this year, when the majority exhibited average values abt. 25, had only an average of 22.915 leaf-teeth, i. e. the same value as for the majority of plants in the preceding year, 1915.

The low figure for number of leaf-teeth in No. 1148 for 1916 however, is to be found in quite another direction than that of climatic conditions.

The table shows the year in which the different plants took root at the places where they stood during the investigations.

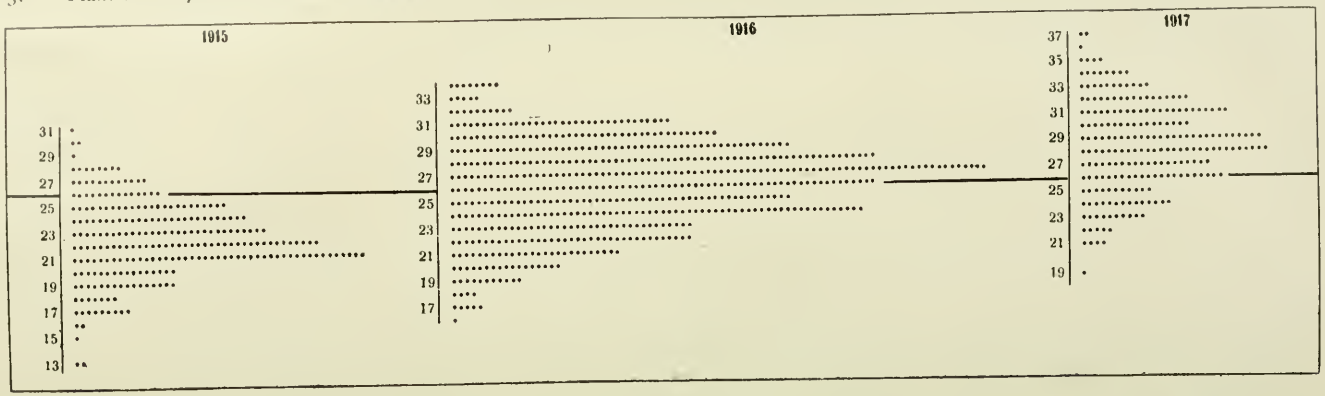
Table I. Plants of the clone "*Mathon 760*", open air.

No.	760		1148		1224			1218			1206 1209 1213	1213	
Site	Row 11		Row 11		Row 6			Row 6			Row 6	Row 6	
Rooted at place of observation	1912		1916		1914			1914			1914	1914	
Examined	1916	1917	1916	1917	1915	1916	1917	1915	1916	1917	1915	1916	1917
n	366	325	599	253	297	662	569	464	462	482	924	526	513
a	**26.210**	**28.025**	**22.915**	**25.731**	**23.970**	**24.600**	**26.090**	**22.647**	**26.286**	**26.029**	**22.689**	**26.669**	**27.016**
σ	± 4.307	± 3.614	± 3.277	± 2.785	± 3.342	± 3.248	± 4.239	± 3.441	± 3.335	± 4.101	± 3.220	± 3.739	± 4.107
P. E. A.	± 0.152	± 0.135	± 0.090	± 0.118	± 0.131	± 0.085	± 0.120	± 0.109	± 0.105	± 0.126	± 0.071	± 0.110	± 0.122
Fl.	± 0.760	± 0.675	± 0.450	± 0.590	± 0.651	± 0.425	± 0.600	± 0.545	± 0.525	± 0.630	± 0.355	± 0.550	± 0.610

No.	1209		1206		1186			1185			1176		
Site	Row 6		Row 6		Row 6			Row 6			Row 6		
Rooted at place of observation	1914		1914		1914			1914			1914		
Examined	1916	1917	1916	1917	1915	1916	1917	1915	1916	1917	1915	1916	1917
n	695	493	583	516	371	409	295	226	579	279	255	615	227
a	**25.368**	**28.152**	**24.792**	**26.990**	**22.291**	**25.756**	**27.512**	**22.898**	**26.064**	**29.065**	**22.458**	**26.046**	**28.379**
σ	± 3.342	± 3.667	± 3.496	± 3.785	± 2.943	± 3.530	± 3.869	± 3.164	± 3.719	± 3.186	± 2.966	± 3.292	± 3.393
P. E. A.	± 0.086	± 0.111	± 0.098	± 0.112	± 0.103	± 0.118	± 0.152	± 0.142	± 0.104	± 0.129	± 0.125	± 0.090	± 0.152
Fl.	± 0.430	± 0.555	± 0.490	± 0.560	± 0.515	± 0.590	± 0.760	± 0.710	± 0.520	± 0.645	± 0.625	± 0.450	± 0.760

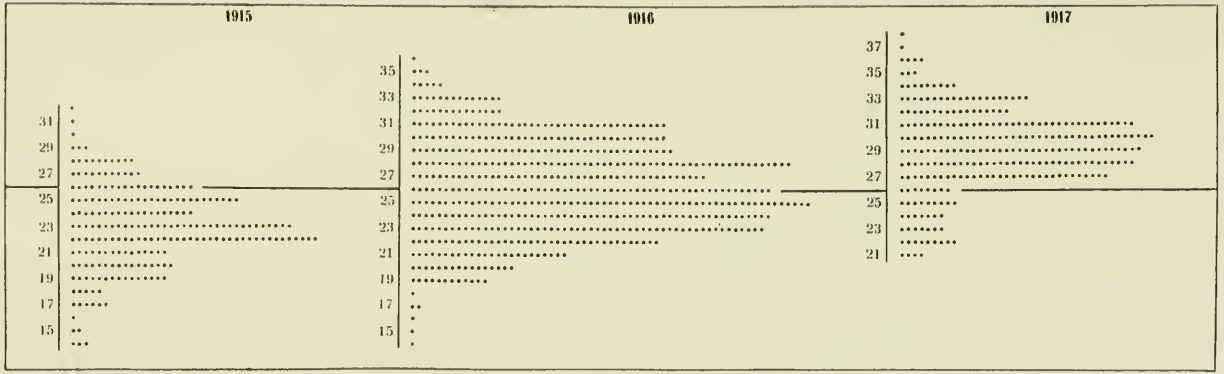
Figs. 3 and 4. — No. of leaf-teeth in 1915, 1916 and 1917 in two plants of the clone "*Mathon 760*".

Fig. 3. — Plant No. 1176 of the clone "*Mathon 760*".



1915	1916	1917
n = 255	n = 615	n = 227
a = **22.458**	a = **26.046**	a = **28.379**
σ = ± 2.966	σ = ± 3.292	σ = ± 3.393
P.E. A. = ± 0.125	P.E. A. = ± 0.090	P.E. A. = ± 0.152
Fl. = ± 0.625	Fl. = ± 0.450	Fl. = ± 0 760

Fig. 4. — Plant No. 1185 of the clone "*Mathon 760*".



1915	1916	1917
n = 226	n = 579	n = 279
a = **22.898**	a = **26.064**	a = **29.065**
σ = ± 3.164	σ = ± 3.719	σ = ± 3.186
P. E. A. = ± 0.142	P. E. A. = ± 0.104	P. E. A. = ± 0.129
Fl. = ± 0.710	Fl. = ± 0.520	Fl. = ± 0.645

All the other plants had remained on the site since 1914 (No. 750 since 1912). No. 1148, however, had been dug up in 1916, and removed to the spot where it was investigated in the same year. There is no doubt that we must in this transplantation seek the cause of the small number of leaf-teeth in 1916 for this plant. In our remaining material, there are also several instances of a low number for the first year in which a plant takes root at the site of investigation. (See for instance Table VII, No. 256).

Probably then, we must presume that the root system is damaged to such a degree by digging up and transplanting, that the supply of water and nourishment does not proceed so completely as is the case when the plant has stood for a long time on the same spot, and had time to develop its root system undisturbed. This supposition would at any rate explain the increase noted in the number of leaf-teeth during the two to three years of observation, though of course it cannot be denied that the hot summer of 1917 may also have helped to bring the number for this year up to a higher level than in the cold summer of 1916.

Table II. — No. of leaf-teeth in the same plant in the years 1915 and 1916. The leaves are classified according to size. The plant was rooted on the site in 1914.

1915							
Size of leaves	I	II	III	IV	V	VI	VII
Length in cm.	< 2	2—4	4—6	6—8	8—10	10—12	> 12
No. of leaves.....	3	55	139	154	81	28	
Average no. of teeth ..	**18.3**	**20.8**	**22.2**	**22.7**	**23.9**	**26.0**	
1916							
Size of leaves	I	II	III	IV	V	VI	VII
No. of leaves		7	79	235	112	23	6
Average no. of teeth ..		**23.3**	**22.8**	**26.3**	**26.7**	**27.2**	**28.8**

Since a large leaf will as a rule naturally have a greater number of teeth than a small one from the same plant, it will be natural to suppose that the increase noted might be due to the fact that the leaves, owing to the better nourishment derived from the

root system as it developes, would on the whole be larger from one year to another. That this alone is not the cause, however, will be seen from the Table II. The leaf material is here divided into groups, and the average number of teeth noted for each group. The figures distinctly show that the number of leaf-teeth within the separate groups was greater in 1916 than in 1915. The increase in the number of teeth in 1916 is thus not due exclusively to increase in the average size of the leaves themselves, but must really also be due to the addition of further points.

IV. The same clone under different external conditions in the same year.

The experiments under this head were carried out with the aid of a glasshouse in the experimental nursery. Some of the plants, here also belonging to the clone "*Mathon 760*", were rooted inside the glasshouse, others outside, part to the north, and part to the south.

Owing to the heat of the sun in the glasshouse, the plants inside naturally grew up under altogether different conditions of temperature etc. than those rooted outside: in other words, they were grown in a different climate.

By means of the glasshouse, it was possible to test the influence of different climatic conditions on the same genotype, the plants inside being compared with those without.

In order to guard against any possible influence of slight differences in the nature of the soil, special precautions were taken. Half the bines from the plants rooted inside the house were drawn out and trained up outside, and similarly, half the bines of the plants rooted outside were trained up inside under glass.

By this method, we could be certain that the bines whose leaves were compared had one common root system, though growing themselves under different climatic conditions.

The results of these investigations, which were carried out in 1916 and 1917, are shown in a highly condensed form in Tables III—IV. Examples are also given in the graphs figs. 5—6.

First of all we may consider the plants rooted inside the glasshouse, of which one half of the bines were inside, the other

Tabel III. Plants af the clones *"Mathon 760"* and *"Færgelunden 227"* (last column only) rooted in glasshouse.

Name and No.	*"Mathon 760"* (No. 1231)				*"Mathon 760"* (No. 1232)				*"Mathon 760"* (No. 1233)				*"Mathon 760"* (No. 1234)			
Site	Rooted in north side of house				Rooted in north side of house				Rooted in north side of house				Rooted in north side of house			
Rooted at place of observation	1916				1916				1916				1916			
Examined	1916		1917		1916		1917		1916		1917		1916		1917	
Picked	inside	outside	inside	outside	inside	outside	inside	outside	inside	outside	inside	outside	inside	outside	inside	outside
n	276	235	134	204	287	134	115	227	279	170	131	211	109	127	168	132
a	**25.188**	**22.111**	**29.104**	**24.804**	**24.798**	**21.284**	**29.852**	**23.004**	**23.681**	**21.206**	**29.885**	**25.957**	**23.404**	**21.606**	**27.720**	**25.091**
σ	±3.164	±3.174	±3.099	±3.170	±3.458	±2.801	±4.102	±3.590	±3.078	±2.814	±3.924	±3.169	±3.053	±3.067	±2.854	±3.125
P. E. A.	±0.120	±0.140	±0.181	±0.150	±0.138	±0.163	±0.258	±0.161	±0.124	±0.146	±0.231	±0.147	±0.197	±0.184	±0.149	±0.183
Fl.	±0.645	±0.700	±0.905	±0.750	±0.690	±0.815	±1.290	±0.805	±0.620	±0.730	±1.155	±0.735	±0.985	±0.920	±0.745	±0.915

Name and No.	*"Mathon 760"* (collection of No. 1231, 1232, 1233, 1234)				*"Mathon 760"* (No. 1342)		*"Mathon 760"* (No. 1343)		*"Færgelunden 227"* (No. 1302)			
Site	Rooted in north side of house				Rooted in south side of house		Rooted in south side of house		Rooted in north side of house			
Rooted at place of observation	1916				1917		1917		1915			
Examined	1916		1917		1917		1917		1916		1917	
Picked	inside	outside	inside	outside	inside	outside	inside	outside	inside	outside	inside	outside
n	977	666	548	774	501	359	509	328	290	256	232	110
a	**24.381**	**21.485**	**29.024**	**24.627**	**22.778**	**21.632**	**21.462**	**20.686**	**29.552**	**26.297**	**26.875**	**24.282**
σ	± 3.223	± 3.021	± 3.593	± 3.469	± 3.200	± 2.698	± 2.514	± 2.919	± 4.522	± 3.735	± 5.314	± 4.174
P. E. A.	± 0.070	± 0.079	± 0.104	± 0.084	± 0.096	± 0.096	± 0.075	± 0.109	± 0.179	± 0.158	± 0.235	± 0.268
Fl.	± 0.350	± 0.395	± 0.520	± 0.420	± 0.480	± 0.480	± 0.375	± 0.545	± 0.895	± 0.790	± 1.175	± 1.340

Table IV. Plants of the clone "*Mathon 760*" rooted in the open air on the north side of the glasshouse.

No.	1227				1228				1229			
Rooted at place of observation	1916				1916				1916			
Examined	1916		1917		1916		1917		1916		1917	
Picked	inside	outside	inside	outside	inside	outside	inside	outside	inside		inside	outside
n	273	74	182	231	113	38	233	228	60		222	92
a	**24.128**	**23.459**	**23.093**	**22.212**	**22.584**	**22.237**	**23.811**	**21.640**	**22.833**		**23.923**	**21.076**
σ	± 3.618	± 4.071	± 4.871	± 2.969	± 2.844	± 3.366	± 4.057	± 3.183	± 2.876		± 3.431	± 2.551
P. E. A.	± 0.148	± 0.319	± 0.244	± 0.132	± 0.181	± 0.368	± 0.179	± 0.142	± 0.250		± 0.155	± 0.180
Fl.	± 0.740	± 1.595	± 1.220	± 0.660	± 0.905	± 1.840	± 0.895	± 0.710	± 1.250		± 0.775	± 0.900

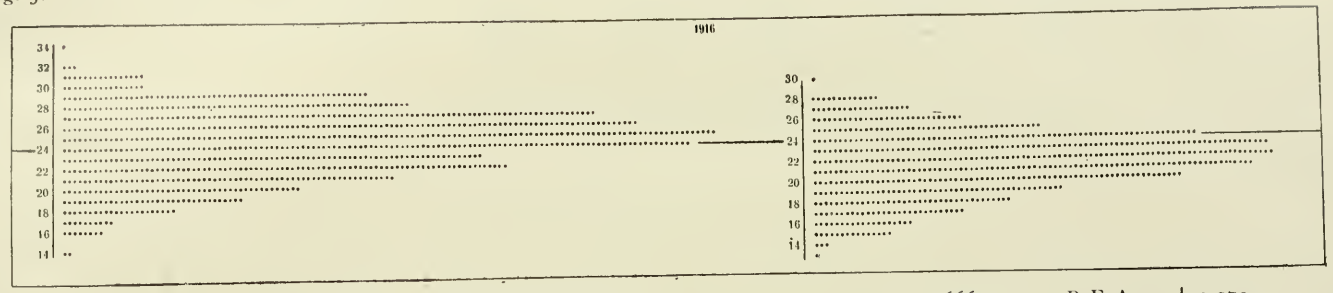
No secondary bines on that part of the plant growing outside the glasshouse.

No.	1230				Collection of No. 1227, 1228, 1229 and 1230					
Rooted at place of observation	1916				1916					
Examined	1916		1917		1916		1917			
Picked	inside	outside	inside	outside	inside	outside	inside	outside	inside	outside
n	10	20	154	184	456	132	791	735		
a	**21.9**	**23.9**	**26.000**	**22.630**	**23.526**	**23.174**	**24.096**	**22.131**		
σ	—	—	± 5.110	± 3.326	± 3.394	± 4.016	± 4.436	± 3.033		
P. E. A.	—	—	± 0.278	± 0.165	± 0.107	± 0.236	± 0.106	± 0.075		
Fl.	—	—	± 1.390	± 0.825	± 0.535	± 1.180	± 0.530	± 0.375		

Figs. 5 and 6. — No. of leaf-teeth in 1916 and 1917 in the plant of clone "*Mathon 760*" rooted in glasshouse. The leaves in the graph on the left are from bines inside the glasshouse, those in the graph on the right from bines trained up outside the glasshouse.

Plants No. 1231, 1232, 1233 and 1234 of the clone "*Mathon 760*".

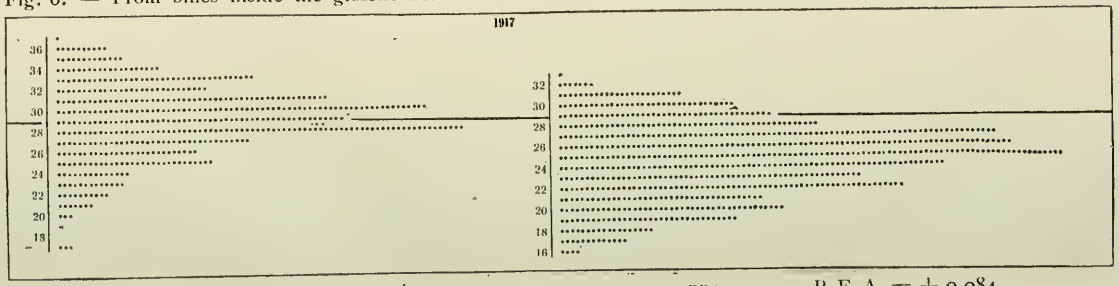
Fig. 5. — From bines inside the glasshouse. From bines outside the glasshouse.



n = 977 P. E. A. = ± 0.070
a = **24.381** Fl. = ± 0.350
σ = ± 3.223

n = 666 P. E. A. = ± 0.079
a = **21.485** Fl. = ± 0.395
σ = ± 3.021

Fig. 6. — From bines inside the glasshouse. From bines outside the glasshouse.



n = 548 P. E. A. = ± 0.104
a = **29.024** Fl. = ± 0.520
σ = ± 3.593

n = 774 P. E. A. = ± 0.084
a = **24.627** Fl. = ± 0.420
σ = ± 3.469

half outside. The figures for these are given in Table III. They show in nice accordance, that in both years of observation, the bines under glass had on an average a greater number of leaf-teeth than those outside. This is shown graphically in figs. 5 and 6, based on figures from plants 1231, 1232, 1233 and 1234, partly for 1916 (fig. 5) and partly for 1917 (fig. 6). The first graph in each figure indicates the number of leaf-teeth inside the glass-house, the second that for outside. In 1916, the average number was abt. 24.381 inside and 24.485 outside; in 1917, the averages were 29.024 and 24.627 respectively.

With the exception of a single plant, No. 1302 (a „wild" Danish plant, brought in from a wood in North Sealand) all the plants noted in Table III belong to the same clone (Mathon 760).

The next series of experiments comprises all plants rooted close outside the glasshouse with half their bines outside, and half inside. Of these plants, some were growing on the north side of the house, others on the south (Tables IV and V).

Table V. Plants af the clone "*Mathon 760*" rooted in the open air on the south side of the glasshouse.

No.	1340		1341	
Rooted at place of observation	1917		1917	
Examined	1917		1917	
Picked	inside	outside	inside	outside
n	142	82	138	39
a	**26.979**	**23.720**	**21.232**	**20.872**
σ	± 3.234	± 3.709	± 3.787	± 3.368
P. E. A.	± 0.183	± 0.273	± 0.217	± 0.364
Fl.	± 0.915	± 1.365	± 1.085	± 1.820

The tables show that, as in the experiments just described, the number of leaf-teeth inside the glasshouse was greater than that outside, though the difference was slighter in the case of the plants rooted inside the house. Furthermore, the figures were altogether lower in the case of the plants rooted outside the glasshouse than for those rooted inside. Why the latter should be affected to a greater degree by the difference of climate than those rooted outside the house, I am unable to say.

Altogether, the experiments dealt with in this section distinctly show that climatic differences are able in an essential degree to influence the number of leaf-teeth in one and the same clone.

V. Comparison of different Clones.

We have seen in the foregoing, that the number of leaf-teeth in one and the same clone could be affected to no slight degree by external conditions, whether of a climatic or other character. The question then is, if it is possible to find any differences at all which are independent of external conditions, as regards the number of leaf-teeth in hop plants of various clones.

Even at an early stage in these investigations it seemed to me that this question must undoubtedly be answered in the affirmative. We therefore only considered it necessary to take a comparatively small number of clones for further examination on this point.

The greatest number of leaf-teeth was found in several American clones, and it is therefore likely that this character may be added to the ones already described — there are not a few of them — in respect of which *Humulus americanus* Nuttall differs from the European *Humulus lupulus* L., according to the investigations of Salmon and Wormald[1]).

The American plants which we have investigated belonged especially to the following varieties: "Oregon Cluster", "Red Vines" and "New York Spaulding English Cluster".

In all of these, high values were found, always more than 30 teeth in the average, even in the case of plants newly transplanted[2]).

I would here refer to Table VI, where the results of the investigations with American hops are noted. The three first plants noted in the table all belong to "Oregon Cluster No. 60", the same clone of which a specimen was examined in 1913 (see graph fig. 2, p. 4). The plant No. 60 in the first column was then (in 1915) rooted inside the glasshouse, and some of its bines

[1]) Journal of Botany, May 1915, p. 132—135.

[2]) An American plant of the "Golden Cluster" variety, of which only a single specimen was examined on a single occasion, gave a markedly lower figure, the average number of leaf-teeth being only 23.867.

Table VI. Plants of the American clones *"Oregon Cluster 60"* (a—d), *"Red Vines 1250"* (e), *"New York Spaulding English Cluster 99"* (f).

Name and No.	a *"Oregon Cluster 60"*		b *"Oregon Cluster 60"*		c *"Oregon Cluster"* 997	d *"Oregon Cluster"* 996, 997, 998	e *"Red Vines 1250"*		f *"New York Spaulding English Cluster 99"*	
Site	glasshouse		open air		open air	open air	open air (row 14)		open air	
Rooted at place of observation	1912		1916		1913	1913	1916		1913	1916
Examined	1915		1916	1917	1913	1915	1916	1917	1920	1916
Picked	inside	outside								
n	74	16	329	66	90	181	108	75	98	52
a	**42.122**	**34.9**	**30.918**	**40.333**	**34.433**	**36.061**	**31.361**	**35.107**	**34.602**	**35.788**
σ	± 4.852	—	± 3.506	± 3.979	± 3.718	± 4.285	± 3.528	± 2.764	± 3.873	± 2.762
P. E. A.	± 0.381	—	± 0.130	± 0.330	± 0.264	± 0.215	± 0.229	± 0.215	± 0.264	± 0.258
Fl.	± 1.905	—	± 0.650	± 1.650	± 1.320	± 1.075	± 1.145	± 1.075	± 1.320	± 1.290

trained up outside. In the bines inside, we find the high average of abt. 42 (abt. 35 outside). In the spring of 1916, the plant was dug up and removed to the open air, where we find it in 1916 and 1917 (second column). And we find also, that the average falls to abt. 31 in 1916, rising again, however, in 1917, to abt. 40.

We have then, quite a considerable number of investigations with the clone "Oregon Cluster 60" and we may therefore claim to have studied the numerical characters of this clone fairly closely. The average figures found are as follows: abt. 34.4 (1913), 36.1, 34.9, 42.1 (1915), 30.9 (1916), 40.3 (1917).

Table VII. Plants of the clone »♂ *No. 4*«.

No.	♂ 4		255	256	
Rooted at place of observation	1915		1913	1912[1]	1916[1]
Examined	1915	1916	1916	1915	1916
n	59	238	125	181	102
a	**14.305**	**15.588**	**18.528**	**18.127**	**15.647**
σ	± 1.204	± 2.458	± 2.840	± 3.230	± 2.013
P. E. A.	± 0.106	± 0.123	± 0.171	± 0.162	± 0.134
Fl.	± 0.530	± 0.615	± 0.855	± 0.810	± 0.660

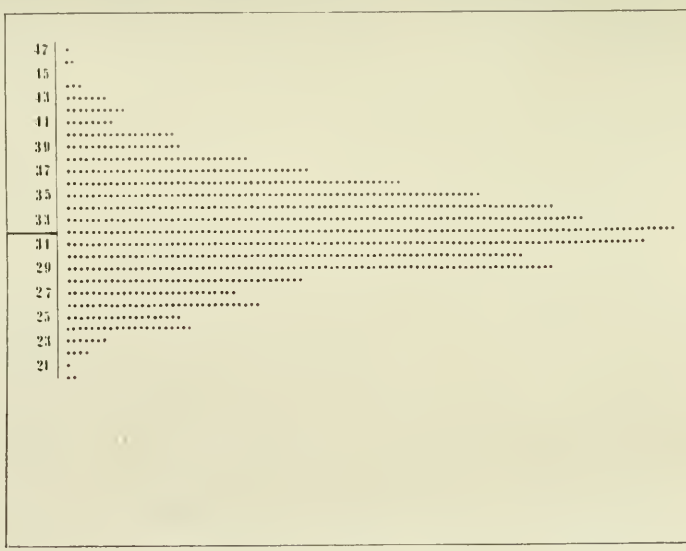
A contrast to the "Oregon Cluster 60" i. e. a clone with a very low number of leaf-teeth, was soon found in a wild-growing Danish male plant from a wood in North Sealand. This plant, which is noted in our journals as "♂ No. 4", was cultivated for several years in our two experimental nurseries, and the values found are shown in Table VII. It will be seen that in no case did the average number of leaf-teeth in this clone exceed 20. The separate averages were: abt. 14.3, 18.1 (1915), 15.6, 15.6 and 18.5 (1916).

Between the lowest average found for "Oregon Cluster 60" and the highest for "♂ No. 4" there is thus a difference of no less than abt. 12.5. As the plants in question were cultivated under highly varying external conditions, especially the "Oregon Cluster" plants, we must say that these experiments have placed

[1]) Note that plant No. 256 was moved in 1916, after having remained on the same site since 1912.

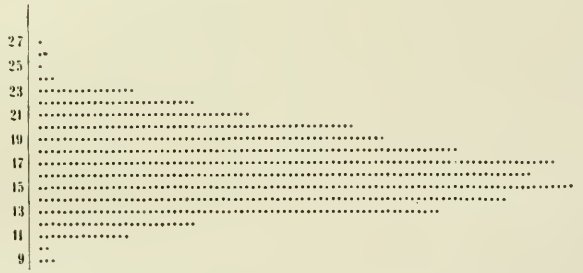
Fig. 7. — No. of leaf-teeth in plants of the clones "*Oregon Cluster 60*" (left) and "♂ *No. 4*" (right).

Fig. 7. — Plants of the clone "*Oregon Cluster 60*".



n = 929
a = **32.260**
σ = ± 4.251
P. E. A. = ± 0.094
Fl. = ± 0.470

Plant of the clone "♂ *No. 4*".



n = 705
a = **16.642**
σ = ± 3.061
P. E. A. = ± 0.078
Fl. = ± 0.390

beyond doubt the fact that there is a difference between the two clones in respect of number of leaf-teeth, which is independent of external conditions.

The accompanying graph (fig. 7) illustrates in a striking manner the difference between the two clones. The graph includes only those specimens of both clones which were cultivated in the open air.

VI. Crossing Experiments.

I will now proceed to describe the last method by which we have studied the number of leaf-teeth in the hop plant, *viz.* by crossing experiments. The idea here was to investigate the number of leaf-teeth in plants raised by crossing a male and a female plant with known characteristic numerical values.

The parent plants selected were specimens belonging to the two clones referred to in the foregoing section, "Oregon Cluster 60" and "♂ No. 4", the former with an unusually high, the latter with a very low number of leaf-teeth. (See Tables VI and VII, p. 17—18).

The crossing was effected in 1912, and the offspring plants were examined in 1915 and 1916. The result of the investigation is shown in Table VIII and graphically in fig. 8. In the last-mentioned figure, all the averages found in the two parent plants under different conditions are noted, as also those for the 19 offspring plants examined in 1915 and 1916. It will be seen that the average figures for the offspring occupy in the main an intermediate position between those of the parents.

The average of all offspring plants does not, however, exactly coincide with the average of the parent plants, but is somewhat nearer that of the mother plant than that of the father. It should in this connection be noted that the population from which the male parent plant was taken (a wood in North Sealand) also includes clones with essentially higher figures than ♂ 4. No. 1302 in Table III, for instance, with averages varying from abt. 24 to abt. 29½ is from the same population as ♂ 4.

Another crossing experiment with an American plant ("New York Spaulding English Cluster No. 99") and ♂ 4 gave a similar result to that just described (fig. 8), but the number of offspring

Table VIII. Offspring plants from crossing "*Oregon Cluster 60*" with "♂ *No. 4*", effected in 1912.

Name and No.	K. 1194	K. 1195	K. 1199	K. 1267	K. 1270	K. 1271	K. 1272	K. 1273	K. 1274	K. 1281
Rooted at place of observation	1914	1914	1914	1915	1915	1915	1915	1915	1915	1915
Examined	1915	1915	1915	1915	1915	1915	1915	1915	1915	1915
n	207	135	96	77	261	54	87	102	109	148
a	**26.242**	**28.711**	**21.250**	**27.714**	**29.448**	**23.315**	**25.287**	**31.902**	**22.963**	**25.919**
σ	± 3.841	± 3.855	± 2.765	± 3.311	± 2.758	± 2.722	± 2.643	± 3.618	± 2.577	± 3.410
P. E. A.	± 0.180	± 0.224	± 0.190	± 0.255	± 0.115	± 0.250	± 0.191	± 0.242	± 0.167	± 0.189
Fl.	± 0.900	± 1.120	± 0.950	± 1.275	± 0.575	± 1.250	± 0.955	± 1.210	± 0.835	± 0.945

Name and No.	K. 1282	K. 1283	K. 1285	K. 1286	K. 1287	K. 1288	K. 1289	K. 1290	K. 1291
Rooted at place of observation	1915	1915	1915	1915	1915	1915	1915	1915	1915
Examined	1915	1915	1915	1915	1915	1915	1915	1915	1915
n	83	208	68	127	42	134	68	71	21
a	**28.506**	**21.702**	**19.912**	**25.992**	**29.929**	**27.567**	**25.118**	**28.535**	**22.476**
σ	± 3.451	± 2.802	± 2.460	± 3.178	± 3.887	± 3.024	± 3.619	± 3.918	—
P. E. A.	± 0.256	± 0.131	± 0.201	± 0.190	± 0.405	± 0.176	± 0.296	± 0.314	—
Fl.	± 1.280	± 0.655	± 1.005	± 0.950	± 2.025	± 0.880	± 1.480	± 1.570	—

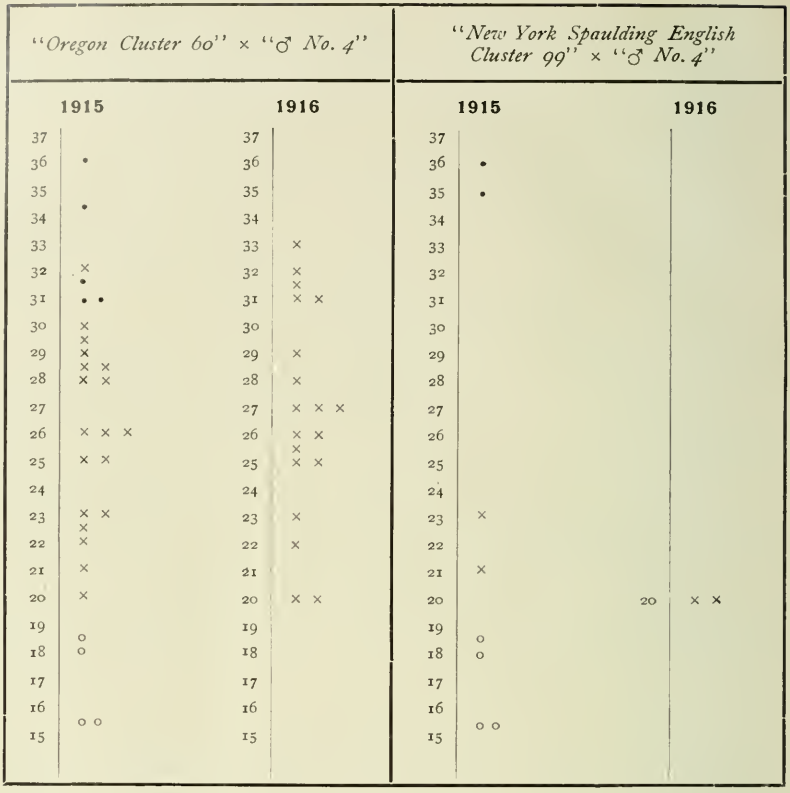


Fig. 8. — Average no. of leaf-teeth in offspring plants produced by crossing "*Oregon Cluster 60*" with "♂ *No. 4*" and "*New York Spaulding English Cluster 99*" with "♂ *No. 4*". • indicates the mother plants, ○ the father plants, and × the offspring.

plants is so small that there is no reason to describe this experiment further.

Unfortunately, owing to lack of space and other considerations, we have not been able to carry on the investigation further, to F 2 and subsequent generations, but must be content with noting that the F 1 plants in the main exhibited such intermediate numerical values as might be expected to arise from the crossing of two highly dissimilar genotypes. The crossing experiments must therefore be said to support the view advanced in the previous section, that the difference between the two clones is independent of external conditions.

VII. Summary.

The present paper deals with a quantitative character in the hop plant, *viz.* the number of teeth in the margin of the leaves. The results of several experiments are noted, and serve to show that although this character is largely affected by environment, certain differences, and these quite considerable, are nevertheless discernible in the number of leaf-teeth in different hop clones.

18. March 1918.

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COMPTES-RENDUS

DES TRAVAUX

DU

LABORATOIRE CARLSBERG

14ME VOLUME.

No. 3

COPENHAGUE

EN COMMISSION CHEZ H. HAGERUP

IMPRIMERIE DE THIELE

1919

Prix: 65 Øre.

ON THE NON-MENDELIAN INHERITANCE IN VARIEGATED PLANTS.

BY

Ö. WINGE.

The investigations of recent years as to the heredity of variegated plants have brought to light certain new facts which necessitate a revision of the views hitherto maintained with regard to the nature of non-mendelian inheritance.

The works of Correns (1902, 1903, 1908, 1909) and Baur (1907, 1908a and 1908b, 1909, 1911) led to the idea that the qualities which distinguish variegated forms as apart from others (in the categories *aurea*, *albomaculata*, *albomarginata*, *chlorina*, *variegata*, the entirely yellov varieties[1]) not capable of living on, and the entirely white ones[2]) similarly incapable are transmitted in different ways, some of the varieties exhibiting mendelian segregation, when the divergent leaf-colouring is as a rule recessive as to the self-coloured green, while others do not follow the mendelian laws.

These last, viz. the *albomaculata* forms of *Mirabilis Jalapa* (Correns 1908), *Antirrhinum majus* (Baur 1911), *Primula sinensis* (Gregory 1915) and the *albina* form of *Pelargonium zonale* (Baur 1908b) naturally called for the supposition that it is not the cell-nucleus which is the bearer of the peculiarities in question, but the cytoplasm or the plastids therein contained.

The *albomaculata* forms of the species: *Mirabilis Jalapa*, *Antirrhinum majus* and *Primula sinensis* appear, from the investigations made, entirely alike, only the mother plant being decisive for the appearance of the offspring, while the father plant has no

[1]) I propose to term these *citrina*.

[2]) I would suggest that these be called *albina*.

effect at all with regard to the character in question. Only in the *albina* form of *Pelargonium zonale* do we find an exception, the two reciprocal crossings between the divergent and a self-coloured green plant giving the same result, viz. "mosaic plants", i. e. a kind of vegetative segregation takes place at once in F_1, with the result that certain portions of tissue in the F_1 plants become self-coloured green, other parts quite white; this can give rise both to sectorial chimæra and periclinal chimæra forms.

Baur (1908 b, 1911) inclined to the view that in the case of the mentioned *albomaculata* forms, it must be the cytoplasm itself (excl. plastids) which was the bearer of the quality or defect in question, while in *Pelargonium zonale*, it must be the plastids situated in the cytoplasm.

Correns (1909) on the other hand, is disposed to consider that it is in all cases the cytoplasm (not the plastids) which have the hereditary property, as he can hardly believe that there should be any essential difference between *Pelargonium* and the remaining forms — he also expresses some doubt with regard to the possibility that plastids should be able to migrate at all from the pollen tube into the egg cell, which is necessarily implied by Baur's theory. Altogether, Correns rather leaves the question open for future determination.

Gregory, who in his work (1915) on *Primula sinensis albomaculata* shows that this form, in respect of heredity, entirely resembles *Mirabilis Jalapa* and *Antirrhinum majus* (this was already suggested by Baur 1911) states that in his view, it is the plastids themselves which are the cause of the peculiarities in the *albomaculata* forms, and he shows, from microscopic investigation, that there in reality occur both large green chromatophores and smaller, sickly ones without colouring.

In 1917, Ikeno showed (1917 a), in the case of *Capsicum annuum*, that a variegated variety which from the description might, it appears to me, likewise be called *albomaculata*, differed from the above-mentioned *albomaculata* forms, the quality being transmitted not only through the mother, but also through the father. The offspring of a parent pair, of which either both or only the one belonged to the variegated type, always gave as offspring exclusively variegated plants. It was found, however, that the more variegated the parents, or the shoots on which the parent flowers appeared, the more variegated would the off-

spring also be. Often there arose green or almost green shoots on variegated plants, but the offspring of flowers on such shoots were nevertheless always variegated — albeit to a somewhat slighter degree. Offspring plants which were entirely green, or entirely devoid of chlorophyl, were in no case met with during the experiments. — Ikeno states as his view that the generative cell must be accompained by cytoplasm with plastids which are bearers of the hereditary quality in question.

In 1917 I published, in a very brief form, the results of some investigations as to the heredity in *Humulus Japonicus albomaculata*, and showed, that the *albomaculata* character was only transmitted through the mother, as also, that no plants entirely devoid of chlorophyl arose in my experiments. Variegated female plants gave throughout only variegated descendants, without regard to the qualities of the father plant; but never entirely green or entirely white.

These experiments of mine, which were carried out in 1911—1916, I will here describe in further detail, before passing on to theoretical considerations.

Experiments with *Humulus Japonicus* Sieb. et Zucc. var. *albomaculata*.

Humulus Japonicus is, like *H. Lupulus*, a dioecious plant, but differs in biological respects from the latter in being annual. The handsome white-mottled variety is doubtless well-known as a decorative twining plant. *H. Jap.* is distinguished by enormous growth for an annual plant, when grown under favourable conditions. In a sunny glasshouse, with well manured soil, it can in the course of the summer attain a length of 5—6 metres, besides a considerable thickness. Cultivated in pots, on the other hand, it can flower when only 1 metre high. In the white-mottled variety, the leaves are more or less speckled with white, as if they had been sprayed

Fig. 1. Leaf of highly variegated specimen of *Humulus Japonicus albomaculata*. × 1/1.

with lime-wash. (Fig. 1—3). As a rule, the normal green colour predominates, the white portions being smaller, but in the case of the most variegated individuals it will be equally correct to

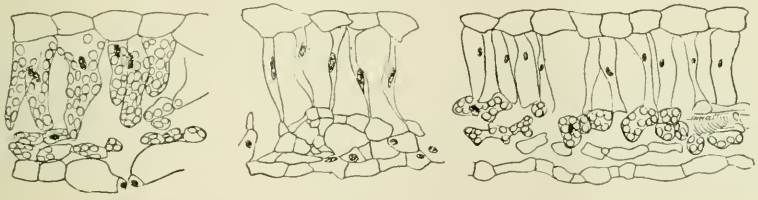
Fig. 2. Leaf of markedly variegated specimen of *Humulus Japonicus albomaculata*. $\times 1/2$.

say that the leaves are white with more or less considerable patches of green. We find however, in the leaves, not only these two colours, but a couple of intermediate shades of a lighter green, according as more or fewer layers of the parenchyma contain clorophyll. All possible combinations seem to be able to arise in this respect (see Fig. 4). The patches of a lighter green,

or quite white, have a sharply cut, angular outline, but are very different in shape, often radiating somewhat in a direction from

Fig. 3. Leaf of very slightly variegated specimen of *Humulus Japonicus albomaculata*. × 1/1.

the base of the leaf to the periphery. In some individuals, the parts without clorophyll are most numerous nearest the margin



a *b* *c*

Fig. 4. *Humulus Japonicus albomaculata*. Leaf sections of *(a)* self-coloured green leaf, *(b)* port. of leaf entirely lack. chloroph., and *(c)* leaf with partially chloroph. parenchyma.

of the leaf, in others nearer the base. The stems and flower clusters also, are often distinctly chloritic; in the case of the stems, however, this is only conspicuous in the young shoot-tips. In highly variegated individuals, we never find large shoots of en-

tirely normal appearance, while on the other hand, no shoots entirely lacking chlorophyll have ever been observed in such. The most slightly variegated individuals have only a smaller number of spots, the colour of which is not white, but pale green, and in such individuals, we may well find apparently normally green shoots. Young shoots in a highly variegated plant may, it is true, appear entirely lacking in chlorophyll, but as the shoot ripens, chlorophyllous parts always appear, and indeed, always also certain parts of the normal green colour.

In the following description, the self-coloured green is noted as *norm. (normalis)* the variegated as *albm. (albomaculata)*.

En 1911, some purchased seeds, partly of the variegated race and partly of the green, were sown out, and the plants growing up from these, standing in a glasshouse, were cross-fertilised in all directions in the course of the summer. The flower clusters concerned were isolated by means of transparent paper bags, closed at the mouth with cotton.

F_1-generation.

In 1912 and 1913 the fruits produced after pollination were sown out, being of the following parentage:

Table I. ♀ *norm.* × ♂ *norm.*

Offspring	
norm.	*albm.*
24	0
12	0
66	0
Summa: 102 *norm.*	0 *albm.*

For this, only one pair of parent plants was used. The three separate experiments indicate only the result of three different sowings; one of these, however, is from a special pollination effected later on.

Table II. ♀ *norm.* × ♂ *albm.*

Offspring	
norm.	*albm.*
2	0
19	0
15	0
36	0

	Offspring	
	norm.	*albm.*
Brought forward	36	0
	15	0
	26	0
	8	0
	19	0
	15	0
	34	0
	13	0
	11	0
	20	0
	27	0
	46	0
	58	0
	68	0
	26	0
	43	0
	74	0
	46	0
	72	0
	48	0
	41	0
Summa:	746 *norm.*	0 *albm.*

For the 23 separate experiments, a total of 14 different normal female plants and 3 variegated males in all were used.

Table III. ♀ *albm.* × ♂ *norm.*

Offspring	
norm.	*albm.*
0	18
0	17
Summa: 0 *norm.*	35 *albm.*

For the 2 separate experiments, 2 variegated female plants and 1 normal male plant were used, this last being the same as that employed in Table I.

Table IV. ♀ *albm.* × ♂ *albm.*

Offspring	
norm.	*albm.*
0	37
0	31
0	17
0	13
0	17
0	28
0	17
0	20
0	22
0	66
Summa: 0 *norm.*	268 *albm.*

For the 10 separate experiments, 9 normal female plants and 2 variegated males in all were employed, these last being 2 of the male plants used for the experiments in Table II.

The F_2-generation.

From the plants grown up in 1913, fruits were collected and sown out in 1914; the result obtained from these will be seen in Table V below.

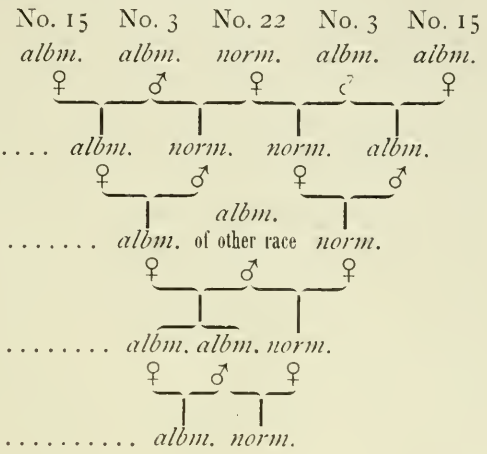
Table V.

	Offspring	
	norm.	*albm.*
♀ *norm.* (*norm.* × *norm.*) × ♂ *norm.* (*norm.* × *albm.*)	14	0
— —	15	0
— —	21	0
— —	28	0
♀ *norm.* (*norm.* × *albm.*) × ♂ *norm.* (*norm.* × *albm.*)	31	0
♀ *norm.* (*norm.* × *albm.*) × ♂ *albm.* (*albm.* × *albm.*)	32	0
♀ *albm.* (*albm.* × *albm.*) × ♂ *norm.* (*norm.* × *albm.*)	0	29

F_3- and later generations.

Finally, in 1915 and 1916, fruits were sown out which had been harvested in 1914 from some of the plants mentioned in Table V. The result will be seen from the pedigree given below. The P-individuals noted in the top line were only three

different, viz. 1 variegated male, 1 variegated female and 1 green female. The experimental numbers of these plants are here noted in the table for the sake of convenience; in the other tables, I have considered it superfluous to note the numbers.



The F_3-generation, which, as will be seen from the table, was sown out in 1915, gave 67 *norm.* and 31 *albm.* derived respectively from normal and *albomaculata* mother plants. The F_4-generation in 1916 embraced only a smaller number of individuals.

Results of my breeding experiments.

As will be distinctly seen from the above tables and pedigree, the mother plant in *Humulus Japonicus* is solely determinative for the appearance of the offspring with regard to colour of the leaves. Not a single deviation occurred among some 1500 plants here noted. Whether the male plant belongs to the *albomaculata* form or is self-coloured green is of no importance at all in determining the leaf colour of the offspring.

It will further be noticed that no *albina* forms ever occurred in the experiments. The *albomaculata* form does not give off either self-coloured green or *albina* forms, and differs thus from all such hitherto investigated forms in which the *albomaculata* quality is only transmitted through the mother plant.

In seeking now to find an explanation of this exclusively maternal heredity, we naturally turn to the possibility that the phenomenon might be due to apogamy. This, however, is not the case, since, as I have shown (1914) pollination of female flowers is necessary for fruit formation. Flower clusters isolated in paper bags only produced fruit when artificial pollination had been carried out, and a cytological investigation showed that the growth of the pollen tube down to the egg cell of the embryo sac proceeded altogether normally. I have not, however, made further investigation as to whether other qualities may be transmitted through the father to the offspring. — The various individuals may differ considerably one from another as regards the power of forming anthocyan especially in female flower clusters, and the stems can also be either green or red-coloured. It would seem as if this power of anthocyan formation is transmitted exclusively through the mother plant, as some small countings showed that the daughter plants, whether their mother had pale green or dark purple hops, were always like those of the mother.

There is then undoubtedly but one possibility remaining, viz. that the *albomaculata* quality (or the normal colour, as the case may be) is transmitted to the offspring exclusively with the cytoplasm of the egg cell, and then either with the plasm itself or with the plastids. Which of these two alternatives is correct we shall consider in the next section. In any case we can say that the quality is not transmitted through the nucleus of the sexual cell, which would necessarily involve normal segregation of the forms — and we can similarly doubtless conclude that the plastids do not migrate with the generative cell through the pollen tube, to the egg cell, and continue their existence there.

With regard to my crossing experiments, I will merely mention one further phenomenon, which I at first considered as of importance for the entire comprehension of the *albomaculata* type, but which I have since ceased to regard as standing in connection with the results obtained.

In 1912, I found among the plants of different parentage sown out, a small number of specimens which were on the whole typically green, but had nevertheless when young, a few small and faint spots of paler green or yellow colour on the first three

pairs of leaves, especially on the second pair. When the plants grew larger, all trace of this disappeared in the following pairs of leaves, and when the lowest pairs had withered away, the appearance was of an entirely normal green. As the spots in question occurred especially in the offspring of a pair of self-coloured green female plants, which had been fertilised by variegated males, I could not disregard the phenomenon, but sowed out again in 1913 a number of fruits of the same crop (1911) as those sown out in 1912. In 1913, I obtained a relatively very large number of these plants which were lightmottled at a juvenile stage. I note here the two most pronounced examples.

Sowing 1912. Observation on young plants.

	Entirely green	Slightly spotted
♀ 22 × ♂ 3 (norm.)(albm.)	304	20
♀ 28 × ♂ 4 (norm.)(albm.)	c. 40	very few

Sowing 1913. Observation on young plants.

	Entirely green	1 pale spot	2—3 pale spots	More pale spots
♀ 22 × ♂ 3 (norm.)(albm.)	1	18	22	17
♀ 28 × ♂ 4 (norm.)(albm.)	5	21	30	12

As the two sowings in 1912 and 1913 are of exactly the same origin, viz. the crop from 1911, it is remarkable that there should be far more juvenile mottled plants in 1913 than in 1912. Of what nature this mottling can be, I do not know. The spots are mostly yellowish-green, angular in outline, and do not appear to be due to the attacks of any fungus growth. The difference between the results obtained in 1912 and 1913 certainly seems to suggest that external conditions affect their occurrence to a very high degree.

These spots, which are as a rule small, few, and faint, and appear only in the young plants, cannot have anything to do with the *albomaculata* quality, which is expressed in the form

of manifold very conspicuous spots large and small, of a pale green colour, or without chlorophyll at all. Of this there can be no doubt. The appearance of the offspring in the following generations is also evidence of the fact. As will be seen from the pedigree p. 9, we have here the very plants No. 22 × ♂ 3, and we find then, that even when the female descendants for several generations are fertilised from variegated plants, the offspring remains consistently green. True, it was not the most pronounced juvenile mottled female plant in 1913 which was selected for propagation of the line, but positive results of the pollination with the variegated male plant should at any rate have been perceptible in 1914. This, however, was not the case at all.

From what has been said above, it will be understood why, in the tables already given for the crossing experiments made, I have stated nothing as to the juvenile mottled individuals.

The hereditary qualities localised in the cell-nucleus, in the cytoplasm and its plastids.

The variously inherited peculiarities in the leaves of variegated plants must, according to Baur (1911) be considered as being differently localised in the sexual cells. Baur extends the division previously advanced by Correns (1909), and gives three distinct categories. They are:

I. The qualities localised in the nucleus — or especially perhaps in the chromatine. All these segregate normally according to the mendelian laws. To this class belong many, mostly recessive, *albina-*. *citrina-*, *aurea-*. *chlorina-*. *variegata-* and *albomarginata* forms.

II. Those localised in the chromatophores. All these segregate vegetatively at once in the F_1-plants — according to the distribution of the chromatophores in the daughter cells (mosaic plants). Here should be placed, according to Baur, the *albina* form of *Pelargonium zonale.*

III. The qualities localised in the plasma. These are inherited, according to Baur, through the mother alone, and to this class belong the *albomaculata* forms of *Mirabilis Jalapa*, *Antirrhinum majus*. *Primula sinensis* and possibly others.

Baur adds, that it is not likely that he has exhausted all existing categories with those above named. And that he is correct in this supposition, is apparent from the investigations of Ikeno and the present writer (both 1917) with *Capsicum annuum albomaculata* and *Humulus Japonicus albomaculata* respectively, with regard to which latter I have given further details above. I also consider it certain that the principle of division for the species already included in Baurs list must be altered somewhat.

The regularly segregating chromatophore qualities we may here simply disregard, as they must of course still be considered as localised in the nucleus of the sexual cell, especially in the chromatine.

On investigating the other forms, which do not segregate according to the mendelian laws, we at once notice that there are evidently two main categories, viz.

1. Chromatophore qualities which involve segregation of *albina* individuals and self-coloured green plants. — To these belong the examples cited by Baur, the *albomaculata* forms of *Mirabilis*, *Antirrhinum* and *Primula*, as also the the "mosaic plants" of *Pelargonium*.
2. Those where only the variegated form reappears in the offspring. To these belong the *albomaculata* forms of *Humulus* and *Capsicum*.

Similarly, the hitherto-known cases of non-mendelian chromatophore qualities can be divided into two categories according to another principle, viz. as follows:

1. Plants where the chromatophore qualities are transmitted only through the mother. — To these belong the *albomaculata* forms of *Mirabilis*, *Antirrhinum*, *Primula* and *Humulus*.
2. Plants whose chromatophore qualities are transmitted through parents of either sex, viz. the *albina* forms of the "mosaic plants" of *Pelargonium* and the *albomaculata* form of *Capsicum*.

When we now come to consider the question as to what can be the foundation of these two divisional principles, there appears to be a very natural explanation. The variegated plant forms, which segregate *albina*- and self-coloured green

forms, must probably have their origin in the chromatophores themselves, whereas those which always produce only variegated offspring must arise from peculiarities in the plasm. This explanation would seem plausible enough, since it is both possible and likely that the normal and the abnormal, bleached chromatophores, even if they are found collected in the fertilised egg cell, will in the course of ontogenesis "segregate" vegetatively. each of the sorts reproducing only chromatophores of their own kind. In the course of the ontogenesis, therefore, there may arise both purely albinotic and purely normal daughter cells, and also mixed albinotic-and-normal. Among the sexual cells formed in the flowers therefore, there can exist the same three categories, which will give rise to an offspring of variegated as well as normal and *albina* forms.

If, on the other hand, we consider the fluid plasm in the cells, it will be natural that no entirely normal should be able to arise as the offspring of an abnormal. We are here not dealing with units, but with masses. The greater the mass of abnormal plasm contained in a cell in proportion to the normal, the greater will be the probability that the chromatophores resting in the plasm will become abnormally marked, or altogether suppressed.

With regard to the second principle for division, maternal or maternal + paternal heredity, we must suppose — and there can hardly be any objection to so doing — that in certain species, no cytoplasm (or none capable of continued existence) accompanies the generative nucleus over into the egg cell on fertilisation, and the result will then be an exclusively maternal inheritance of chromatophore qualities. In others, however, the generative nucleus is accompanied by cytoplasm, and the plasm- or chromatophore peculiarities of the father plant will then also make themselves apparent in the offspring. — From this, it follows that the oft discussed question as to transmission of cytoplasm from the father should not, as has hitherto been believed, be regarded as a question of one or another alternative, but involves the consideration off both, according as we are dealing with one or another species of plant.

As will be seen, I am here in disagreement with Baur (1911) with regard to one important point. He supposed, that the then known *albomaculata* forms with exclusively maternal

inheritance *(Mirabilis, Antirrhinum* and *Primula)* originated in plasmatic peculiarities — in contrast to the "mosaic plants" of *Pelargonium*, which according to Baur's view were due to special chromatophore (plastid) qualities. I, on the other hand, am of opinion that we have only in the *albomaculata* forms of *Humulus* and *Capsicum* quite recently investigated found instances of the heredity of plasmatic peculiarities.

The difference between the "mosaic plants" of *Pelargonium zonale* and between the *albomaculata* forms of *Mirabilis. Antirrhinum* and *Primula* appears to me to be really not a principal. Correns has, by the way, already (1908) expressed the same view, as I have mentioned. If, for instance, we merely suppose that *Pelargonium* has in the fertilised egg cell only a small number of plastids, the others having sligthtly more, then the difference in appearance can be easily explained. Elementary mathematics will tell us that cells with a small number of green and white plastids will, by constant bisection in the course of a few cell generations, possibly even on the first division, give off both purely albinotic and self-coloured green cells in the descendance, and only a very few of a mixed character, later none at all — whereas cells with but a slightly (not greatly) superior number of green and white plastids will only after a several cell generations, when the embryo has reached a considerable size, give off albinotic and self coloured green cells, and will never in the course of the ontogenesis lack cells with mixed content. It should here be borne in mind that it is by no means any extraordinarily great number of cell generations which will be required before a respectable plant has arisen from the divisions of the fertilised egg cell; as a rule probably far less than 100. The different cells in the fully-grown plant will, however, of course have gone through a widely different number of cell divisions, some doubtless not even 20. Still, it is impossible to work out exactly the probability of this vegetative segregation, as long as we do not know with what degree of accuracy the number of plastids is equally divided between the daughter cells. We also, by the way, lack knowledge as to the number of plastids in the cells of the embryo and in the course of the ontogenesis — a point which it would be most interesting to investigate with regard to the variegated plants.

Otherwise, in my opinion, there is only the great difference

of principle between *Pelargonium* on the one hand and *Mirabilis*. *Antirrhinum* and *Primula* on the other, that in *Pelargonium*, on fertilisation, cytoplasm of unimpaired vitality passes over with plastids to the egg cell, together with the generative nucleus, whereas this is not the case with the other forms.

As regards *Primula sinensis albomaculata*, in which Gregory (1915) has so finely shown both normal and abnormal chloroplasts in the somatic tissue, I can thus only support Gregory's view as opposed to that of Baur (Gregory l. c. p. 315) "the existence of normal and chlorotic chloroplasts side by side in the same cell appears to me to afford definite support for the view that the abnormality is localised in the chloroplasts themselves, and is not a function of the cytoplasm as a whole".

Ikeno (1917 a) opines, with regard to *Capsicum annuum albomaculata*, that here also it is the plastids themselves which much be regarded as the bearers of the quality. As will appear from the foregoing, I must, in this plant particularly, regard the plasma itself as the seat of heredity in the direction concerned, as we never here obtain *albina* forms, nor selfcoloured green forms from variegated forms. It agrees in this respect with *Humulus Japonicus*, save that in the latter, no cytoplasm is transmitted to the egg cell together with the generative nucleus, which makes itself apparent in the exclusively maternal form of inheritance. In *H. Jap.* I have not succeeded in discovering plastids at all in the cells without chlorophyll. These are strikingly smaller, and, as it were, shrunken.

We can, then, I believe, set up the following schema with regard to hereditary variegation of leaves. Category I is merely a repetition of that given in Baur's schema (1911) under the same term.

I. The characters are situated in the cell nucleus, especially in the chromatine. Such forms segregate according to the mendelian laws, and are often recessive as to the self-coloured green. To this class belong:
   1) The *albina* forms of *Melandrium* and *Antirrhinum*. Recessive. (Baur 1907).
   2) The *citrina* form of *Antirrhinum* and *Pelargonium*. Not recessive. Green × *citrina* gives *aurea*. (Baur 1907, 1908 a).
   3) The *chlorina* form of *Mirabilis* and *Urtica* (Correns

1902, 1903, 1908, 1909) and of *Antirrhinum* and *Aquilegia* (Baur 1910, 1911). All recessive.

4. The *variegata* form of *Mirabilis* (Correns 1902, 1903, 1908, 1909) and *Aquilegia* (Baur 1911). Both recessive.
5. The *albomarginata* form of *Lunaria*. Recessive. (Correns 1908).

II. The characters are not situate in the cell nucleus, but in the cytoplasm — and then either in the plasm itself or in the plastids. No mendelian segregation.

A. The characters are situate in the plastids, and *albina* forms and normal forms can therefore arise.

a. The generative nucleus is on fertilisation accompanied by cytoplasm of unimpaired vitality with plastids, which pass over into the egg cell. The quality is transmitted through both parents. — In this class, we have at present only the "mosaic plants" of *Pelargonium zonale* (Baur 1908 b).

b. No cytoplasm with plastids passes over into the egg cell on fertilisation. Consequently only maternal inheritance. To this class belong the *albomaculata* form of *Mirabilis* (Correns 1908), *Antirrhinum* (Baur 1911) and *Primula* (Gregory 1915).

B. The characters are situate in the plasm, wherefore no albina forms or normal forms are produced.

a. The generative nucleus is accompanied on fertilisation by cytoplasm of unimpaired vitality, which pass over into the egg cell. The quality is transmitted through both parents. To this class belongs only the *albomaculata* form of *Capsicum* (Ikeno 1917 a).

b. No cytoplasm passes over into the egg cell on fertilisation. Therefore only maternal inheritance. To this class belongs only the *albomaculata* form of *Humulus* (Winge 1917 and present paper).

It is quite remarkable that what we know of examples of non-mendelian variegation is in all cases restricted to *albomaculata* forms, since the "mosaic plants" of *Pelargonium* can also doubt-

less, as I have mentioned, be reckoned among these. This does not, however, exclude the possibility that other forms may subsequently be found. We know as yet no instance of mendelian *albomaculata* forms, yet it is surely possible that such may exist. There is still much which remains to be investigated with regard to the heredity of variegated plants, and much must be investigated before we have the question clear. The theory here set forth must in all essentials be regarded as a working hypothesis, the correctness of which must be demonstrated or disproved by the results of future work.

True or false heredity.

I will now merely add a few words concerning the real nature of the hereditary conditions here concerned.

Divergent opinions have often been expressed as to whether we can properly speak of true heredity when dealing with non-segreating qualities. We often hear that it is not a question of inheritance, but of "direct transmission" and the question has been compared with that of the hereditarily transmitted tuberculosis, which, it is maintained, may be due to infection of the sexual cells themselves with bacteria. Ikeno, in a subsequently published note to his work on *Capsicum* (Ikeno 1917 b) deals with the questions of inheritance and mutation. He states that since his variegated *Capsicum annuum* individuals originated from normal plants, it is apparently, no doubt, a mutation which has taken place, but as the mutation, in his opinion, here concerns the plastids alone, the term is incorrect, "because this term is restricted to such a case where there is either a gain or a loss of certain factors or perhaps, as some authors think, where there is a change in their configuration". Ikeno suggests the term "pseudomutation". With regard to the variegated *Capsicum* plants which arose in his cultures, he writes "Thus we have to deal with no genetical change at all."

I must admit that I am quite unable to follow Ikeno in these considerations, which have too much philosophy about them and too little of reality. Qualities which have their material foundation in such portions of the sexual cells as belong to the organism itself, — which portions further propagate in the organism formed of sexual

cells and can pass over anew to its sexual cells — such qualities must be termed hereditary, whether localised in the nucleus, plastids or plasma — and I would suggest that the definition of true heredity be formulated in accordance herewith.

It must absolutely be maintained that mendelian segregation and new combination are not identical with the idea of "true heredity", but are only a form, albeit no doubt the commonest form, for hereditary transmission. Otherwise, of course, we might equally well maintain that the qualities localised in the chromosomes were likewise not hereditary, since the chromosomes also pass, by "direkte Uebertragung" to the offspring. But it is perhaps one of the great advances made by the researches of late years that we have realised that all inheritance is by no means mystical and incomprehensible; that it is indeed transmitted to the offspring by direct transition of material elements.

Tuberculosis, in the case quoted above, is naturally not inherited in the proper sense, but transmitted as something not really belonging to the organism in question at all. From organisms whose sexual cells contain poisonous matter, or are markedly ill-nourished, or in other physiological respects affected by external conditions, the offspring can no doubt (falsely) "inherit" the quality in question from the parents, but these cases also fall, — as with tuberculosis — outside the definition. It seems to be possible to distinguish sharply between true and false heredity in all cases.

2. Nov. 1918.

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COMPRESSIBILITY OF AQUEOUS SOLUTIONS OF URETHANE.[1]

BY

THEODORE W. RICHARDS AND SVEN PALITZSCH.

In previous papers from Wolcott Gibbs Laboratory[2] a marked relation between compressibility and surface tension, as far as pure liquids are concerned, has been pointed out. Moreover, it has been possible to express this relation approximately by the empirical equation $\beta \cdot \gamma^{4/3} = k$, where β represents the compressibility and γ the surface tension. In mixtures of two liquids, however, the conditions are less simple and the relation between surface tension and compressibility more obscure[3]), especially if water is one of the two. On account of the very complicated internal structure of these mixtures, their behavior could not be expected to be expressed in any formula as simple as that holding true for unmixed substances. Nevertheless they offer a problem of considerable interest.

As early as in 1886 W. C. Röntgen and J. Schneider[4]) measured and compared the two properties of many aqueous solutions of inorganic salts, which showed, without exception, a higher surface tension and a lower compressibility than pure

[1]) The measurements in the present and following paper were carried out at the Wolcott Gibbs Memorial Laboratory during the winter 1915—16, when Sven Palitzsch was at work at Harvard University as Fellow of the American-Scandinavian Foundation and having leave of absence from Carlsberg Laboratorium, Kobenhavn; they may be considered as the beginning of a research intended to be carried out on the volume condition in solutions of organic substances, especially those important for physiological Chemistry.

[2]) Journ. Amer. Chem. Soc. **30**, 11 (1908); Zeitschr. physik. Chem. **61**, 451 (1908).

[3]) A. Ritzel: Zeitschr. physik. Chem. **60**, 319 (1907).

[4]) Ann. d. Phys. u. Chem. **29**. 165 (1886).

water[1]). They found a significant dependence without being able to express it by any equation. In the case of cane-sugar, which in aqueous solution decreases the compressibility[2]), measurements of surface tension showed values sometimes greater, sometimes less than pure water[3]). The very few measurements published about colloidal solutions indicate that both their surface tension and their compressibility decrease at the same time. (Compare the following paper).

Aqueous solutions of two solid organic acids, namely mono- and trichlor- acetic acid, were found by K. Drucker[4]) to have a much smaller surface tension than water, normally decreasing with the concentration, whereas the compressibility at first decreases and then increases with rising concentration. In this latter respect these solutions resemble several mixtures of liquids, ethyl alcohol and water, acetic acid and water, and dichloracetic acid and water. Whether or not this peculiarity corresponds to irregularities of the other properties of the involved substances, was not investigated.

In interpreting results concerning single components, one of us has shown in several publications that the compressibility of a pure substance is probably in part contingent upon the internal pressure, the external pressure required to compress the substance to a given extent being greater the more the molecules are previously compressed by molecular pressure. The more complicated the system, the less likely would this general rule be to hold true. On the other hand, Tammann[5]) has denied the existence of any relation between the surface tension of solutions

[1]) Max Schumann, dealing with very dilute aqueuos solutions of potassium-, calcium, ammonium- and strontium-chlorides, observed, it is true, a compressibility greater than that of water (Ann. d. Phys. u. Chem. **31**, 14 (1887)), but Röntgen and Schneider (*Ibid.* **31**, 1000 (1887)), thoroughly repeating his experiments, could not verify his conclusion.

[2]) G. de Metz: Ann. d. Phys. u. Chem. **41**, 663 (1890), E. Cohen and R. B. de Boer: Zeitschr. physik. Chem. **84**, 77 (1913).

[3]) G. Quincke: Ann. d. Phys. u. Chem. **35**, 582 (1888), Grunmach: *Ibid.* (4) **3**, 660 (1900), F. Plato: Wissenschaftl. Abh. d Kaiserl. Normal-Eichungskommission, 2. Heft, 123 (1900) (quoted from G. Wiegner: Kolloid-Zeitschr., **8**, 127 (1911)), J. Traube: Ber. d. deutsch. chem. Ges. **42**, 86 (1909), J. L. R. Morgan and H. E. Woodward: Journ. Amer. Chem. Soc. **35**, 1249 (1913).

[4]) Zeitschr. physik. Chem. **52**, 641 (1905). On account of its title we first noticed this paper after having completed the urethane measurings.

[5]) Ueber die Beziehungen zwischen den inneren Kräften und Eigenschaften der Lösungen. Leipzig 1907, p. 178.

and the internal pressure (»Binnendruck« computed from thermal expansion), and W. C. McC. Lewis[1]) has found that in aqueous solutions the surface tension and the internal pressure change in the same direction, except with volatile substances and colloidal solutions. The importance of a decision between the apparently conflicting opinions is emphasized by the fact that a theory based on the relationship between internal pressure and surface tension has been recently employed by Traube[2]) to explain many different physiologico-chemical and physico-chemical effects.

Seeking to obtain more light on these questions, we thought it worth while to study several properties of aqueous solutions of a highly soluble substance, neither dissociated nor associated in aqueous solutions, in order to obtain results as easy to interpret as possible.

To represent such substances we have chosen urethane being not dissociated and according to Meldrum and Turner[3]) likewise not associated in aqueous solutions. Besides it dissolves very freely with increase of the total volume, and its solutions possess considerably less surface tension than water. Hence it presents an unusually interesting case, different from most others which have been investigated.

The compressibility of urethane was the chief property to be studied. In order to obtain as much light as possible, rough estimations were made also of density, surface tension and viscosity.

The outcome of the experiments, of which the experimental details will be described in the following pages, was that *the compressibility at first decreased rapidly and after that increased slowly with rising concentration*, while the surface tension continually decreased at first rapidly and then very slowly. It can be seen from the tables I and II as well as from the figures I and II.

In the figure II the four properties: compressibility, surface tension, solution volume, and viscosity, are plotted on appropriate scales.

These four curves at first sight appear to have very little relation to one another; but a reasonable interpretation of them

[1]) Zeitschr. physik. Chem. **74**, 640 (1910).
[2]) Pflügers Archiv **132**, 511 (1910); **140**, 109 (1911).
[3]) Journ. Chem. Soc. (London) **97**, 1805 (1910).

TABLE 1. **Compressibility of urethane solutions** at 20.0° under a pressure of 100 to 300 megabars.

	Concentration		Density D	Weight of solution used W	w	Compressibility $\times 10^6$
	g/100 g water	g/100 g solution				
Water	0.00	0.00	0.99823	—	—	43.25
A	2.28	2.22	1.00105	29.410	3.1970	42.49
B	20.88	17.27	1.01945	29.628	2.9024	39.17
C	37.22	27.12	1.03050	28.206	2.7236	38.93
D	61.63	38.13	1.04125	29.695	2.8618	39.32
E	89.63	47.25	1.04950	29.397	2.8653	40.00
F	127.35	56.01	1.05620	29.500	2.9221	40.86

TABLE 2. **Solution volume and surface tension of urethane solutions.**

	Concentration		Solution volume	Surface tension	
	g/100 g solution	molecular		Drop number	dyn/cm
0	0.00	0.00		52.8	72.6
A	2.22	0.25	(0.877)	65.2	59.0
B	17.27	1.98	0.881	87.5	44.7
C	27.12	3.14	0.886	96.3	41.1
D	38.13	4.46	0.893	101.5	39.4
E	47.25	5.57	0.898	103.1	39.1
F	56.01	6.66	0.904	105.8	38.6

TABLE 3. **Viscosity of urethane solutions.**

Concentration		Density D	Time of flow seconds: t	$\frac{t}{t_o} \cdot 100$	$\frac{t}{t_o} \cdot \frac{d}{d_o} \cdot 100$
g/100 g water	g/100 g solution				
0	0	0.9982	331.5	100.0	100.0
10.03	9.12	1.0096	395.0	119.2	120.6
20.03	16.69	1.0188	454.0	137.0	139.8
40.10	28.62	1.0320	554.5	167.3	173.0
80.21	44.51	1.0470	711.0	214.5	225.0
100.28	50.07	1.0517	777.5	234.5	247.1
125.49	55.65	1.0559	851.5	256.9	271.6

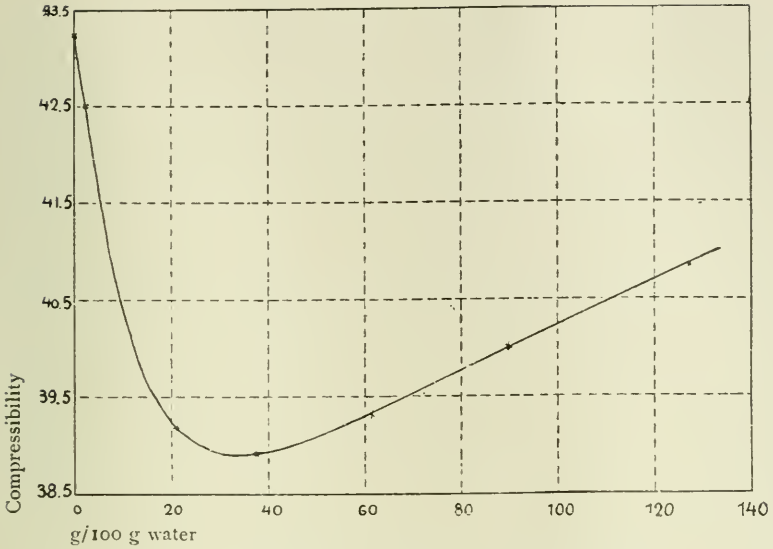

Fig. 1. Compressibility of aqueous urethane solutions.

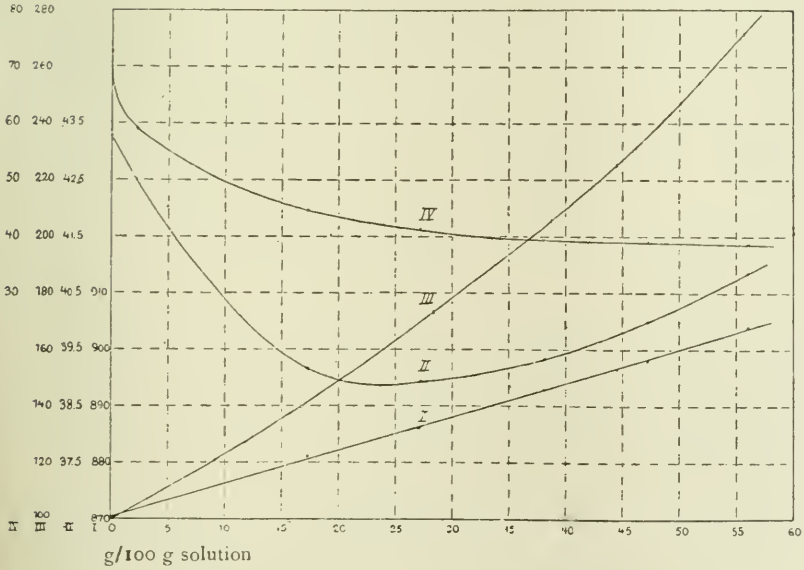

Fig. II. Solution volume (I), compressibility (II), viscosity (III) and surface tension (IV) of aqueous urethane solutions.

is, nevertheless, not impossible. This interpretation depends upon the remarkable and extraordinary properties of pure water, which obviously enter essentially into every one of the curves (especially on the left-hand branches of them, where water is in excess), and is entirely in accord with the modern view that water is not pure hydrol, but contains also varying amounts of di-hydrol and tri-hydrol, according to circumstances.

In view of the fact that most text-books on Physical Chemistry pass over the polymerization of water with but scant reference, a brief resumé may be apropos. The notion that water contains a dissolved polymer is usually ascribed to Röntgen[1]); but it was undoubtedly conceived before. In a work published in 1884 Harold Whiting states very clearly that water even after melting must contain "dissolved particles of ice"[2]). He points out that in freezing water there may be somewhere between 25 % and 38 % of dissolved ice, and in boiling water, somewhere between 17 % and 28 %; and that the true expansion of water (apart from the contraction caused by the melting of the dissolved ice), instead of being only 4 %, is probably from 8 % to 10 % between 0° and 100°. These figures are remarkably like those proposed sixteen years later by W. Sutherland[3]). Röntgen's paper elaborates these ideas, and Sutherland elaborated them still further, with the addition perhaps of some assumptions of less validity. Ten years later the Faraday Society conducted a highly interesting symposium on the subject of the constitution of water[4]), at which papers were read by Walden, Guye, Bousfield and Lowry, Sutherland and Nernst. At the end of the discussion the chairman, Professor James Walker, spoke as follows:

»Often in a discussion of this nature we have great diversity of opinion, but here there seems to be little divergence, although the experimental material which has been worked with is so exceedingly diverse in the different cases. I should think, as a

[1]) Wiedemann's Annalen, **45**, 91 (1892).

[2]) Harold Whiting, "A new theory of cohesion applied to the thermodynamics of liquids and solids," pp. 70—71. Cambridge, Mass., published by W. H. Wheeler. This interesting monograph, which contains much food for thought, seems not to have received the attention which it deserves.

[3]) Phil. Mag., **50**, 464 (1900). W. Sutherland calculated the amount of tri-hydrol to be 37.5 %, by weight, at 0°, and 21.7 % at 100°.

[4]) Trans. Faraday Soc., **6**, 71—123 (1910).

result of this discussion, one will soon find, even in the text-books, that whilst ice is trihydrol, and steam monohydrol, liquid water is mostly dihydrol with some trihydrol in it near the freezing-point, and a little monohydrol near the boiling-point."

The more recent highly interesting work of Bridgman at high pressures[1]) entirely supports the conclusion that the abnormality of water is due to polymerization, and the shift of the minimum volume toward lower temperatures with increasing pressure is entirely in accord with the rational assumption that pressure diminishes the proportion of "dissolved ice". At 2500 atmospheres indeed so little of the polymer appears to remain that the liquid behaves in a normal way like other liquids, possessing no minimum of volume or maximum of density with decreasing temperature. One other circumstance in Bridgman's results is at first puzzling, namely, that the well known minimum of compressibility at 50° is not thus shifted toward lower temperatures, but with increasing pressure is wiped out where it stands at 50°. As Bridgman says, this fact is not consistent with the assumptions of Röntgen and Sutherland. On the other hand, it seems to us entirely consistent with that of Bousfield and Lowry, which assumes that above 50° monohydrol (possessing like trihydrol a greater bulk than dihydrol) becomes important in quantity or concentration. One can hardly doubt that pressure in every instance tends to diminish the quantity of the more bulky form of substance, since this is the verdict of the universal and uncontradicted theorem of Le Chatelier.

The conclusion as to the nature of liquid water offers the basis of the explanation of the four curves presented in the present paper.

Of the four curves given, that representing compressibility was the main object of the present work, and is the most interesting, since it possesses a well marked minimum; hence this will be considered first. As regards the left-hand branch of this curve: the remarkable decrease in compressibility with increasing concentration of urethan may be ascribed rather to the change in the water than to any direct effect of the comparatively small quantity of urethane added, especially as the latter must have a compressibility in the liquid state not very far different from that of water. From a single case such a conclusion might be

[1]) Proc. Amer. Acad., **47**, 544 (1912).

hazardous; but so far as we know all aqueous solutions show thus a marked diminution in compressibility in dilute solutions with increasing concentration, even when the dissolved substance is more compressible than pure water. A striking example is the well known behavior of the alcohol and water mixture; in spite of the fact that alcohol has nearly twice the compressibility of water, the addition of alcohol to water diminishes the compressibility of the solution until (at 20°) 20 % of alcohol has been added[1]). Nearly if not quite all other liquids show a much more nearly additive relationship in solutions. One can hardly escape the conclusion that the first and most striking effect of dissolving anything in water is to dissociate part of the tri-hydrol present, thus diminishing the compressibility. This diminution is entirely parallel to the diminution of compressibility of water with rising temperature, a phenomenon which disappears at high pressure because at high pressures the tri-hydrol present must have been already largely dissociated by the pressure itself.

Is not a similar cause the reason for the generalization of Tammann, at least as regards aqueous solutions, namely: "Das Volumen einer Lösung verhält sich Temperaturänderungen gegenüber, wie das Volumen des einen höhern Druck unterworfenen Lösungsmittels"[2]). The dissolved substance must be supposed to have the same effect as pressure; it partially dissociates the associated molecules, and the resulting material must, therefore, act similarly in the two cases.

These considerations entirely explain the behavior of the left-hand branch of the compressibility curve. The behavior of the right-hand branch may be supposed simply to be due to the possession of a somewhat greater compressibility on the part of urethane than is possessed by di-hydrol, and although the curve does not extend so far because of the saturation of the solution, one has a right to conclude that the compressibility of liquid urethane at 20° (supposing that it could exist in the liquid state at this temperature) would be not far from that of water.

The upward departure from a straight line, visible at the right-hand end of the curve, may reasonably be ascribed to the increasing presence of monohydrol, since we know that when

[1]) Pagliano: Cimento (3) **27**, 209 (1890); see also Landolt-Börnstein-Roth, Physikalisch-chemische Tabellen, p. 62 (1912).

[2]) Zeitschr. Physik. Chem., **74**, 629 (1910).

water is dissolved in other substances very appreciable amounts of it may be present as monohydrol[1]).

It will be noted that the other cases in which water enters, alluded to earlier in this paper, are all consistent with this interpretation[2]). The compressibility curve, then, offers no difficulties.

The "solution-volume" curve, exhibits no unexpected peculiarities if the interpretation of Bousfield and Lowry is accepted. The term "solution volume" is one proposed by them for the excess of volume of a solution over that of the water entering into it. In the table above this excess has been calculated for one gram of dissolved substance. From the table it appears that in a dilute solution the solution volume of urethane is 0.877, and that this increases slowly, until in a 56 % solution it attains the value 0.904. Both of these numbers are larger than the specific volume of solid urethane and less than the probable specific volume of liquid supercooled urethane at that temperature.

The specific volume of solid urethane, computed by interpolation from H. Block's[3]) values, is, at 20° C., 0.8704, and the hypothetical specific volume of the supercooled liquid at 20° (by extrapolation from Block's values from 46° to 57°) is not less than 0.923. It appears, then, that although solid urethane expands on being dissolved, this expansion must be due primarily to the act of melting (*i. e.* to the breaking up of the crystal structure), not to the act of solution. As a working hypothesis, the larger value will be taken for the substance in solution, since this is probably more like the liquid than like the solid. Evidently when the liquid is thus mixed or dissolved to a dilute solution in much water, it must be supposed to cause a great contraction in the water, like that produced when, for example, alcohol is dissolved in water; and from the curve it is clear that this contraction per unit of urethane is greatest at first, gradually decreasing as the concentration of the solution increases, as in the case of alcohol and water. For the highest concentrations the curve turns faintly upward; may this not be due here also to the increasing appearance of the more bulky monohydrol as the concentration of the water diminishes? In brief, the performance

[1]) G. Bruni and M. Amadori: Trans. Faraday Society, **5**. 290 (1909).
[2]) De Metz, Drucker, Tammann, Lewis, *loc. cit.*
[3]) Zeitschr. physik. Chem., **78**, 412 (1912).

of the solution volume curve is precisely what one would expect if water contained an appreciable amount of trihydrol in dilute solutions and an appreciable amount of hydrol (monohydrol) in concentrated solutions, with dihydrol present throughout.

Turning now to the surface tension curve, we note that at first the falling off is very marked, and that later (in solutions containing 40 % or more of urethane from which probably most of the trihydrol has been eliminated), the surface tension decreases far less rapidly but more regularly. Here again the two markedly different branches of the curve must be ascribed to the changing condition of the solvent. The part of the curve between 40 % and 56 % (representing a solution influenced as little as may be by changing association, if we may judge from the other curves) manifests the general principle applicable to pure substances concerning the relation between compressibility and surface tension. Here the surface tension decreases about 1.25 % where the compressibility increases about 2 % between these limits, a relationship which is remarkably in accord with the equation found for pure liquids, $\beta \cdot \gamma^{4/3} = K$. Thus this relationship seems to hold in solutions as well as with pure substances, when abnormal side-reactions have been eliminated.

There remains only the viscosity curve to be considered. Evidently urethane is, as one would expect, far more viscous even than trihydrol, so that even at first the addition of the dissolved substance causes an increase in viscosity, the added urethane more than compensating in this respect for the withdrawal of some of the trihydrol. That this assumption is not unreasonable is shown by the enormous increase in viscosity from 100 to nearly 250 in a 50 % solution. The diminishing presence of trihydrol, therefore, manifests itself only in the departure from a straight line as shown by the fact that $\frac{dx}{dc}$ steadily increases. In the neighborhood of 40 % again the curve seems to be nearly linear. Hydration of the urethane may be the cause of the upward trend at the extreme right of this curve. But in any case viscosity is not very closely related to the other properties, since it must be considered to depend upon the size of molecules and their upward configuration quite as much as on the internal pressures holding them together.

Experimental part. All the determinations of compressi-

bility were carried out under precisely the same conditions and in the same way as usual at the Wolcott Gibbs Memorial Laboratory[1]), of course with the improvements gained in recent years.

The glass piezometer used was precisely like the one pictured in the article just mentioned[2]). The pressure was measured 15 minutes after applying it, the temperature was 20.00°, and compressibilities were calculated as average values for the pressure range from 100 to 300 megabars, as usual[3]) with the aid of the equation:

$$\beta_{100-300} = \frac{(w' - w'_1) \cdot D}{13.562 \cdot W \cdot 200} + 3.96 \cdot 10^{\div 6}$$

where β = average compressibility between 100 and 300 megabars.

W = weight of solution used.

D = density of solution at 20°.

w' = weight of mercury corresponding to the change of volume caused by the pressure within the range 100 to 300 megabars.

w'_1 = the like weight when the whole jacket is filled with mercury. For the piezometer used this weight amounts to 0.1269.

13.562 = density of mercury at 20° under a pressure of 300 megabars.

$3.96 \cdot 10^{\div 6}$ = the average compressibility of mercury between 100 and 300 megabars.

(The difference between the compressibilities of the glass of the piezometer and the mercury was found to be $1.48 \cdot 10^{\div 6}$, and consequently the compressibility of the glass $3.96 \cdot 10^{\div 6} - 1.48 \cdot 10^{\div 6} = 2.48 \cdot 10^{\div 6}$.)

Concentrations in the tables are given as g urethane per 100 g water (Table I and fig. 1), as g-mol. urethane in 1000 cm³ solution (Table II), and as g urethane in 100 g solution (Tables I and II). The concentrations, expressed in the last terms, are plotted as abscissae in fig. II, where the data for the different proportions are plotted as ordinates.

The solution-volume of the dissolved substance is com-

[1]) Journ. Amer. Chem. Soc. **34**, 974 (1912) etc.

[2]) *Ibid.*, **34**, 975 (1912).

[3]) *Ibid.*, **34**, 980 (1912).

puted from the difference of the volume of the solution and of the water and calculated for 1 g urethane.

Surface tension was measured only roughly, because the object was not to obtain exact values, but merely to ascertain how the tension varies with the concentration. Traube's stalagnometer was employed[1]); the number of drops was 52.8, when the apparatus used was filled with water. In Table II are given the number of drops with the several solutions and the surface tension in dynes/cm, computed from the ratio between the drop number of the water and of the solution, multiplied by the surface tension of water[2]), 72.6 dynes/cm, by the density of the solutions, and finally divided by 0.9982. In this term the surface tension is plotted as ordinates in fig. II.

Viscosity was measured by an Ostwald's viscosimeter of glass. The amount of liquid used in the apparatus was 25 cm^3, and the time of flow of pure water was 331.5 seconds. A thermostat kept the temperature constant at 20.00° to within 0.02°. In Table II are recorded the times of flow in seconds as well as these values divided by the time of flow of pure water, and, furthermore, this rate multiplied by the ratio density of the solution to that of water of same temperature. In fig. II the viscosities, expressed in the last term, are shown graphically.

The substance employed (urethane or ethyl carbaminate, $NH_2—CO—O—C_2H_5$, molecular weight 89.1) was beautifully crystallized, and its solution gave no turbidity with added silver nitrate[3]). The melting point was 48.0°—48.2°; compared with 48.5°, H. Block[4]), 47.9°, Bridgman[5]), and 48.14°, Tammann[6]). When dissolved it was nearly neutral; tested with litmus paper and phosphate mixtures[7]) as liquid of comparison, the hydrogen ion concentration was found to be about $10 \div 6$. On account of the volatility of the substance the nitrogen content could not be determined by Kjeldahl's method, nor a possible water content by drying in a vacuum desiccator.

[1]) The much more exact apparatus of J. L. R. Morgan for determining drop weights was not needed in this approximate determination.

[2]) Richards and Coombs, Journ. Amer. Chem. Soc., **37**, 1674 (1915).

[3]) Dumas: Liebigs Annalen, **10**, 284 (1834).

[4]) Zeitschr. physik. Chem., **78**, 397 (1912).

[5]) Proc. Amer. Acad., **51**, 114 (1915).

[6]) Kristallisieren und Schmelzen, p. 229. Leipzig 1903.

[7]) Comptes-rendus du Lab. de Carlsberg, **8**, 42 (1909).

Summary.

The compressibilities of aqueous solutions of urethane were measured at 20.0° over a pressure range from 100 to 300 megabars. With rising concentration the compressibility decreases rapidly from $43.25 \cdot 10^{\div 6}$, the compressibility of pure water, to $38.91 \cdot 10^{\div 6}$, the compressibility of a solution having 34 g urethane in 100 g water. From this point the compressibility increases, at first slowly; at the highest measured concentration, 127.35 g urethane for 100 g water, the compressibility is $40.86 \cdot 10^{\div 6}$. The surface tension, the specific volume and the viscosity were likewise measured; no minimum resembling that of compressibility was found by these latter measurements, and it is shown that none is to be expected. The bearing of the results on the theory of Harold Whiting and others, ascribing polymerization to water, is emphasized.

It is a pleasure to express our thanks to the Carnegie Institution of Washington for the pecuniary support in this investigation.

November 1918.

COMPRESSIBILITY OF AQUEOUS SOLUTIONS OF CASEIN AND PEPTON.[1])

BY

SVEN PALITZSCH.

To understand many of the problems of physiological chemistry, a knowledge of molecular forces in solutions has been of greater and greater importance. Surface tension has, especially in recent times, been considered as an expression of these forces. However, in the case of the more or less colloidal solutions in question this property is frequently very difficult to measure; in most solutions of proteins, split proteins and similar substances the surface tension is lowered with rising concentration, and the substance will therefore, according to Gibbs' rule, accumulate, sometimes to a very high degree, in the surface, where it furthermore often coagulates, forms films, etc.

For that reason it would be advantageous to investigate a property which, like the surface tension, might be expected to give information as to the molecular conditions, and which could be measured without the difficulties arising from the peculiar state of the surface. Such a property is the compressibility, which gives an expression of the state of all the molecules in the solution, not only thoses in the surface. As mentioned in the foregoing paper, the relation between the surface tension and the compressibility does not seem to be very plain; but whether or not the relation between the two properties is clear and simple, the compressibility is nevertheless such an elementary property that it may be able to furnish valuable information as to the internal structure and behavior of physiologically interesting solutions.

As regards colloidal solutions, very few measurements have

[1]) Compare the note relating to the foregoing papers title.

been published, and nobody has, as far as I know, measured both surface tension and compressibility of the very same solutions. On account of the very uncertainly defined composition of the colloids employed, a comparison of data from different papers would hardly be of any value.

However, it ought to be noticed that G. de Metz[1]) in 1890 measured the compressibility of liquids polarizing light when rotated. They included a non-solidifying lime solution and a 10 percent solution of gum arabic in water, and both of them showed a compressibility less than that of water. Very noticeable too it is indeed, that a 2 percent lime solution, immediately after dissolving, was found to be more compressible than water and during solidifying was less and less compressible, approaching the compressibility of water. The surface tension of these solutions is far from being well determined. G. Quincke[2]) found, both with a highly diluted gelatine solution and a 20 percent solution of gum arabic, surface tensions considerably less than that of water; as against this, L. Zlobicki[3]) obtained only with gelatine solutions a less, but with a 2 percent solution of gum arabic, a markedly greater tension than with water.

In 1891—1898 Barus[4]) examined the compressibility of proteins dissolved in water; but the method used was too primitive to furnish really quantitative results.

The first accurate measurements were carried out by L. J. Henderson and F. N. Brink[5]), who in 1908, dealing with gelatine solutions, found that at 20.0^0 the absolute compressibility of

a 10 percent gelatine solution is $39 \cdot 10 \div 6$ within 100 to 500 megabars
a 0.2 „ „ „ „ $41 \cdot 10 \div 6$ „ „ „ „ „
water „ $42 \cdot 10 \div 6$ „ „ „ „ „

Accordingly they found a decreasing compressibility; the surface tension probably decreases at same time.

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[1]) Ann. d. Phys. u. Chem. **41**, 663 (1890); the original paper in Russian language was published in Abhandl. d. math. Abt. d. Naturforscherges. d. Univ. Odessa, **9**, 139 (1889); the paper in Ann. d. Phys. u. Chem., **35**, 497 (1888) contains nothing about compressibility, although it is often quoted as if this were the case.

[2]) Ann. d. Phys. u. Chem., **35**, 582 (1888).

[3]) Bull. Acad. Sc. Cracovie, July 1906, p. 488; quoted from Wo. Ostwald: Kolloidchemie, 2. Aufl. p. 226 (1911).

[4]) Sillim. Amer. Journ. of Science (4) **156**, 285 (1898); the data are quoted in detail in Wo. Ostwald: Kolloidchemie. 2. Aufl. p. 147—148 (1911).

[5]) Amer. Journ. of Physiology **21**, 248 (1908).

The research just mentioned was carried out at the Chemical Laboratory of Harvard University by means of the apparatus used by Theodore W. Richards in his many investigations on compressibility. With a similar apparatus and in the same way, but of course using the improvements gained since that time, the compressibilities of aqueous solutions of casein and of pepton have just been measured.

As for the casein solutions, the concentration not only of casein but also that of hydrogen ions was varied. It is a well known fact that pure casein is insoluble in water but can be dissolved in acid and in alkaline solution; this behavior is expressed more rationally by saying that casein is insoluble at its isoelectric point corresponding to the hydrogen ion concentration, $2.5 \cdot 10^{\div 5}$, as measured by L. Michaelis and H. Pechstein[1]), and that it is soluble both at higher and at lower hydrogen ion concentration (consequently also at neutral reaction), although in somewhat different degree. It could perhaps be supposed that casein at strongly alkaline reaction behaved like a "true" (molecularly dispersed) solution, while with the hydrogen ion concentration approaching the isoelectric point it changed, becoming more and more like a suspensoidal solution until the casein was finally precipitated. Now, since suspensions have nearly the same properties as water when the particles are large, the compressibility could be supposed to approach that of water at the same time as the hydrogen ion concentration approached the point of precipitation. Quincke's experiment with solidifying gelatine also supports this assumption.

Furthermore, it is known from other solutions that the surface tension changes with the hydrogen ion concentration (or the hydroxyl ion concentration, which amount to the same thing) a fact also indicating a difference in compressibility of dissimilarly reacting casein solutions.

Two series of solutions were now examined; the hydrogen ion concentration of the one corresponded to p_H.[2]) equal to about 10, and that of the other to p_H. equal to about $6 \cdot 7$. The former solutions are strongly alkaline and colour, for instance phenol-

[1]) Biochem. Zeitschr. **47**. 260 (1912).

[2]) Comptes-rendus du Laboratoire de Carlsberg, **8**. 4 (1909); Biochem. Zeitschr. **21**, 131 (1909).

phthalein deep-red, while the latter are weakly acid and closer to the isoelectric point.

As can be seen from table I and II, *the compressibility, solutions of identical concentration being compared, is very nearly the same in the weakly acid and in the alkaline solutions.* Accordingly the compressibility was not found to be different at different hydrogen ion concentration, as could be supposed according to the above mentioned reasoning.

TABLE I. **Compressibility etc. of acid casein solutions.**

g casein for 100 g water	g NaOH for 100 g water	Density D	Solution volume	p_H	Weight of sol'n used W	w	Compressibility $\cdot 10^6$	Viscosity $\frac{t}{t_o} \cdot 100$
1.481	0.029	1.00220	0.735	6.82	28.424	3.0982	42.58	145
3.053	0.059	1.00625	0.738	6.71	27.488	2.9538	42.11	211
5.522	0.107	1.01245	0.738	6.65	28.379	3.0015	41.77	430
8.597	0.166	1.01985	0.738	6.62	27.763	2.8643	41.03	1450
9.983	0.195	1.02265	0.743	6.60	28.765	2.9193	40.56	—

TABLE II. **Compressibility etc. of alkaline casein solutions.**

g casein for 100 g water	g NaOH for 100 g water	Density D	Solution volume	p_H	Weight of sol'n used W	w	Compressibility $\cdot 10^6$
5.009	0.201	1.01190	0.729	9.98	28.280	2.9884	41.71
8.006	0.321	1.01955	0.729	9.85	29.183	3.0086	41.08
10.012	0.402	1.02495	0.725	9.77	27.771	2.8128	40.51

TABLE III. **Viscosity of alkaline casein solutions.**

g Casein for 100 g water	p_H	Time of flow in seconds	$\frac{t}{t_o} \cdot 100$
0.0	—	331.5	100
2.0	10.08	551.6	166
4.0	10.01	1010	305
6.0	9.94	2212	667
8.0	9.85	7400 7200	about 2200
10.0	9.77	30 000 28 200 26 400	about 8400

The viscosity was also measured; the data, recorded in Table III, show that the viscosity at first rises slowly and then at 6 to 10 percent extraordinarily rapidly [1]). No similar behavior was found with the compressibility, as the data obtained (see Tables I and II) showed *a compressibility decreasing continually, and at least as regards the more concentrated solutions, approximately linearly.*

Further, it can be noted that — as also pointed out by E. Laqueur[2]) — the viscosity of the acid solutions increased considerably more slowly than is the case with the alkaline solutions. As above mentioned, the compressibility was nevertheless the same.

The solution volume (0.729—0.743), recorded in table III, is the sum of the volume of casein and of sodium hydroxide; on account of the formation of sodium caseinate and of the dissociation of this salt, it is without purpose to subtract the volume of sodium hydroxide from this sum. The solution volume of casein in the solid state is $\frac{1}{1.318} = 0.759$ cm^3 [3]). During solution a slight contraction may then take place.

As representing hydrolyzed proteins, pepton "Roche", prepared from silk-fibrin, was examined. Probably it is a tetrapeptid consisting of 2 molecules of glycocoll, 1 molecule of alanin and 1 molecule of tyrosin (regarding further details see the following experimental part).

The compressibility decreases continually with rising concention, and much more rapidly than is the case with casein solutions.

As can be seen from Table V, the solution volume is a good deal less than that of casein. The surface tension decreases first rapidly and then very slowly, and the viscosity decreases continually.

Accordingly, casein, gelatine as well as pepton diminish the compressibility, but in very different degree.

These three proteins have certainly a less surface tension than water, even if it is not measured exactly, and consequently the compressibility and the surface tension change in the same

[1]) A similar result was obtained by H. Chick and C. J. Martin: Kolloid-Zeitschr., **11**, 102 (1912). The hydrogen ion concentration of the solutions used was not measured.

[2]) Hofmeisters Beiträge z. chem. Phys. u. Path., **7**, 279 (1906).

[3]) H. Chick and C. J. Martin: Kolloid-Zeitschr., **12**, 69 (1913).

TABLE IV. **Compressibility of pepton solutions.**

g pepton for 100 g water	Density D	Weight of solution used W	α	Compressibility · 10^6
0	0.99823	—	—	43.25
3.986	1.01125	29.174	3.0487	41.29
7.978	1.02325	29.328	2.9059	39.71
11.970	1.03470	29.730	2.8054	38.33
16.032	1.04555	29.524	2.7088	37.67

TABLE V. **Hydrogen ion concentration, solution volume, surface tension and viscosity of pepton solutions.**

g pepton for 100 g water	Solution volume	p_H	Surface tension		Viscosity	
			Drop number	dyn/cm	Time of flow	$\frac{t}{t_0}$ · 100
0	—	—	52.8	72.6	200	100
3.986	0.665	6.34	67.0	57.9	226	113
7.978	0.670	6.40	69.0	56.9	246	123
11.970	0.671	6.43	72.0	55.1	280	140
16.032	0.673	6.45	74.0	54.1	295	148

direction, not — as is the case of inorganic salts — in opposite directions.

Experimental part. Methods, apparatus and calculations used were quite the same as mentioned in the foregoing paper.

The *casein* employed was prepared by C. A. F. Kahlbaum according to Hammarstens method; such casein is entirely free from fat and has a fairly constant composition. The casein was weighed and dissolved in weighed quantities of water and tenth normal sodium hydroxide and then filtered. The alkaline solutions contained 10 cm^3, and the acid solutions 4.8 cm^3 tenth normal sodium hydroxide to one g casein.

The *pepton* used was from F. Hoffmann — La Roche et Co., Basel-Grenzbach; according to Fischer and Abderhalden[1]) "Pepton Roche" contains 15.30 % N, 6.05 % H and 52.43 % C. The preparation employed for the present research contained 14.90 % N and vas hydrolyzed to such an extent that 59 % of

[1]) Sitzungsber. d. Kgl. Preuss. Acad. d. Wiss. **30**, 574 (1907).

the total nitrogen could be determined by formol titration[1]) as amino-acid nitrogen.

The *hydrogen ion concentration* was measured electrometrically. The differences of p_H. of the various casein and pepton concentrations are due to the dilution and the consequent increase in dissociation, and not to any addition of acid nor base.

The *viscosity* of the most concentrated casein solutions changed very rapidly. The three values, recorded in Table III, of the 10 percent alkaline solution were observed with quite the same liquid and each time of flow immediately after the other. The time elapsed between the preparation of the casein solution and the measuring of their viscosity being not observed and being not the same in every case, the values obtained are not absolutely exact, but still sufficiently good for the comparison they are used for here. The latter remark also applies to the pepton solutions, the viscosity of which was measured merely by a Traubes stalagmometer.

Summary.

The compressibility of casein solutions decreases with rising concentration and very nearly to the same extent in weakly acid and in strongly alkaline solutions. The compressibility of pepton solutions also decreases with rising concentration, only somewhat more. The concentration being 10 g for 100 g water, the compressibility of acid casein solution is $40.6 \cdot 10^{\div 6}$, of alkaline casein solution $40.5 \cdot 10^{\div 6}$, and of a pepton solution $39.0 \cdot 10^{\div 6}$. The hydrogen ion concentration, the solution volume and the viscosity were also measured.

In concluding, it gives me great pleasure to acknowledge my indebtedness to Professor, Dr. Th. W. Richards for the favour to use the facilities for this research at Wolcott Gibbs Memorial Laboratory, and for his kind interest during the work. To the Carnegie Institution of Washington I am indebted for the pecuniary support in this research.

November 1918.

[1]) Comptes-rendus du Laboratoire de Carlsberg, **7**, 1 (1907); E. Abderhalden, Handbuch der biochemischen Arbeitsmethoden, **6**, 262 (1912).

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COMPTES-RENDUS

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JOHS. SCHMIDT:

RACIAL-INVESTIGATIONS.

III. EXPERIMENTS WITH *LEBISTES RETICULATUS* (PETERS) REGAN.

BY

JOHS. SCHMIDT.

I. Introduction.

The purpose of the experiments about to be discussed was to contribute information on the rather obscure question whether or to what extent quantitative racial characters are hereditary.

The tropical american Cyprinodont *Lebistes reticulatus* (Peters) Regan[1]) was employed in the experiments. I have previously used this little aquarium-fish in experimental investigations, namely, for the purpose of demonstrating the importance of environment upon the number of organs (dorsal rays).

Lebistes reticulatus is, like so many of its relations, viviparous, and under favourable conditions the female brings into the world at intervals of about 4 weeks a considerable number of young. The young possess at birth the full complement of vertebræ, dorsal rays, etc., which fact is immediately recognisable.

The investigations fall into two groups, of which the first helps to elucidate the importance of outer factors (temperature) upon the number of dorsal rays. The second proposes to investigate whether hereditary (i. e. dependant upon inner factors) differences in the number of the rays of this fin may be proved to exist in different individuals. Before I proceed to discuss the experiments, I should draw attention to the fact that the number of dorsal rays in *Lebistes reticulatus* varies from 5 to 8. By far the most usual number is 7.

[1] C. Tate Regan: A Revision of the Cyprinodont Fishes of the Subfamily *Poeciliinæ* (Proceedings of the Zoological Society of London 1913, vol. II, p. 977—1018, London 1913).

[2] Johs. Schmidt: Constancy Investigations continued, Racial Investigations no. 2 (Comptes Rendus du Laboratoire Carlsberg, **13**, no. 1, Copenhague 1917).

The countings in question were carried out by myself; the calculations by Frk. KIRSTINE SMITH D. Sc. The fitting up of the aquaria with constant temperatures was directed by Hr. Dr. phil. Ö. WINGE.

II. Outer factors.

The first experiments in this connection I have already previously (loc. cit.) discussed. The principle was to vary the temperature for the same pair of parents from one period of gravidity to the other and then determine the number of dorsal fin rays in the various broods of offspring.

In the beginning I had no means of maintaining a constant temperature in the aquaria and was therefore compelled to limit myself to stating that the animals in the experiments were kept at a "low", "medium" and "high" temperature, in which "medium" temperature was c. 6° above "low" and c. 3° below "high". "Low" temperature was generally equivalent to c. 19° C., varying between c. 17° and c. 23°.

In the experiments 5 different pairs of *Lebistes reticulatus* were used The following table gives the number of rays in the parents and in addition the average number of rays in the various broods of offspring.

	Parents	Offspring (Averages of the various broods)
1)	♂ **7** × ♀ **7** . . . **7.600**	— 7.000 — ***6.658*** — ***6.921*** — **7.200**
2)	♂ **8** × ♀ **8** . . . 7.341	— ***6.844***
3)	♂ **8** × ♀ **7** . . . 7.140	— ***6.838***
4)	♂ **6** × ♀ **6** . . . 6.867	— 6.868 — ***6.417***
5)	♂ **6** × ♀ **6** . . . 7.000	— ***6.500*** — ***6.660***

In explanation: heavy type means that the young in question were developed at a "high" temperature, italics, that development took place at a "low" temperature. The remaining numbers refer to young developed at "medium" temperature. In addition, I refer to the detailed description, loc. cit. As both the table drawn up there and the averages quoted above show, there was considerable variation in the total of rays in the different broods of offspring of identical parentage. With each pair of parents the difference was expressed in this manner, that the average number of rays was larger in offspring which had developed at high temperature than in offspring the

development of which had taken place at low temperature. To this rule not one exception was found.

In all my later experiments this result has been confirmed. I will content myself with discussing a single one of the later experiments, which, technically speaking, has the advantage over the preceding ones in that the temperature in the aquaria could be kept constant, there being a fluctuation of only one tenth of a degree ($\pm$ 0.1°). The two parents had respectively 7 and 5 rays in the dorsal fin. The experiment took place partly at 25°, partly at 18°. The three first broods were produced at 25°, following which the parents were maintained at 18°, from the day the third brood was born until the birth of the fourth brood. After this the temperature was raised again to 25°, at which degree the development of the last broods of young took place.

The result of the experiment is given in Table I.

Table I. Number of rays in offspring of the same pair of parents at various temperatures.

Number of rays in the offspring	Number of specimens					
	25°	25°	25°	18°	25°	25°
	Brood 1 born 15/5 1918	Brood 2 born 10/6 1918	Brood 3 born 6/7 1918	Brood 4 born 21/9 1918	Brood 5, 6, 7 born 25/10, 21/11, 21/12 1918	Brood 1, 2, 3, 5, 6, 7
7	8	13	29	6	51	101
6	1	2	3	13	4	10
5				1		
n	9	15	32	20	55	111
a	**6.889**	**6.867**	**6.906**	**6.250**	**6.927**	**6.910**
σ	$\pm$ 0.333	$\pm$ 0.352	$\pm$ 0.296	$\pm$ 0.550	$\pm$ 0.262	$\pm$ 0.288
P. E.	$\pm$ 0.075	$\pm$ 0.061	$\pm$ 0.035	$\pm$ 0.083	$\pm$ 0.024	$\pm$ 0.018
Fl.	$\pm$ 0.375	$\pm$ 0.306	$\pm$ 0.177	$\pm$ 0.415	$\pm$ 0.129	$\pm$ 0.092

Thus we see, that whilst the broods developed at 25° had an average of 6.91 rays, the average number of rays fell to 6.25 at 18°.

As all experiments in this connection have given a similar

[1] Difference between the average of the specimens developed at 25° and at 18° is 0,660 and the probable error of this difference being 0.085.

result, it may be taken as proved that the number of rays in the offspring's dorsal fin is affected to a considerable degree by the temperature to which the mother is subjected whilst in a state of gravidity. Remarkable besides is the great difference in the duration of gravidity at the different temperatures: at 25^0 pregnancy lasted circa 1 month, at 18^0 more than 3 months.

III. Inner factors.

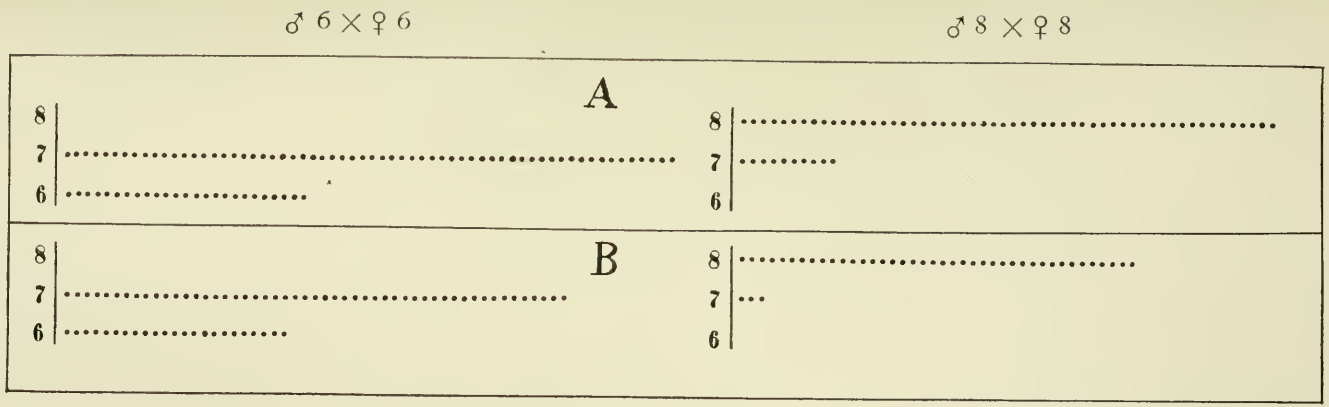
The object of the experiments about to be discussed was to investigate whether hereditary differences in the number of dorsal fin rays could be proved to exist. The principle of the experiments was to maintain different pairs of parents in the same environment, and observe whether the offspring were different as regards the number of rays.

The specimens which were employed in the experiments were selected from two races with which since 1915 successful selection experiments had been carried out partly towards a high, partly towards a low number of dorsal rays. The parents had, respectively, both 8 and both 6 rays in the dorsal fin.

In each case the specimens were kept at a constant temperature, viz. 25^0. In addition, in order to secure uniform environment, the specimens whose offspring should be compared were placed in the same aquarium, separated only by a trelliswork of thin glass tubes. In other words they lived in exactly same body of water, maintained at a constant temperature. The aquarium contained no plants, but a continuous stream of atmospheric air bubbled through the water.

The experiment falls into two series, A and B. In series A there were employed partly ♂ 269 and ♀ 270 (each with 6 rays), partly ♂ 267 and ♀ 268 (each with 8 rays). For series B there were employed partly ♂ 274 and ♀ 273 (each with 6 rays), partly ♂ 276 and ♀ 275 (each with 8 rays). All the experiment fish in series A were kept in the same aquarium which stood at the side of the one in which all the fishes belonging to series B were placed.

From the appended Table II. and from the graph on page 5 one remarks that there was in both series a very great difference in the average number of rays in the offspring of fishes



Number of rays in the dorsal fin in offspring of 4 pairs of parents, all maintained at 25°. — Graphical description of the same experiment as given in Table II.

The figures give the number of rays; each dot represents one offspring. The two upper graphs refer to Series A; the two lower to Series B of the experiment. The two graphs on the left represent offspring of parents having 6 rays in the dorsal fin, the two on the right represent parents with 8 rays.

It is to be seen that in each series the offspring is different in spite of the environment being identical.

Table II. Number of rays in offspring of 4 different pairs of parents at a constant temperature of 25°.

Number of rays in the offspring	Series A		Series B	
	♂ 269 × ♀ 270 both **6** rays	♂ 267 × ♀ 268 both **8** rays	♂ 274 × ♀ 273 both **6** rays	♂ 276 × ♀ 275 both **8** rays
	brood 1, 2, 3, 4 born $^{23}/_{9}$, $^{17}/_{10}$, $^{9}/_{11}$ and $^{4}/_{12}$ 1918	brood 1, 2, 3, 4 boan $^{27}/_{9}$, $^{23}/_{10}$, $^{19}/_{11}$ and $^{17}/_{12}$ 1918	brood 1, 2, 3, 4 born $^{29}/_{9}$ $^{24}/_{10}$, $^{21}/_{11}$ and $^{18}/_{12}$ 1918	brood 1, 2, 3 born $^{29}/_{9}$ $^{26}/_{10}$ $^{26}/_{11}$ 1918
8		54		40
7	62	10	51	3
6	25		23	
n	87	64	74	43
a	**6.713**	**7.844**	**6.689**	**7.930**
σ	$\pm$ 0.455	$\pm$ 0.366	$\pm$ 0.466	$\pm$ 0.258
P. E.	$\pm$ 0.033	$\pm$ 0.031	$\pm$ 0.037	$\pm$ 0.027
Fl.	$\pm$ 0.165	$\pm$ 0.154	$\pm$ 0.183	$\pm$ 0.133

with 8 and with 6 rays, namely in the first series **1.131** (P. E. Diff. = 0.045) and in the second **1.241** (P. E. Diff. = 0.045). This difference cannot be due to difference in environment because the fishes swam in the same aquarium, indeed in the very same water at a constant temperature and with regular ventilation. The conditions with regard to uniformity were in my opinion the most favourable possible and I am forced to the conclusion, that the difference proved to exist in the number of dorsal rays in offspring of parents with respectively 6 and 8 rays is of hereditary (genotypical) nature.

IV. Summary.

The investigations here treated which fall into two groups: 1) Experiments in which the same mother was exposed to different environments in different gravidity-periods and 2) experiments in which different mothers were exposed to the same environment, have thus succeeded in elucidating these rather complicated questions.

It has been shown that the number of organs can be very susceptible to environment but that this fact cannot — under

suitable experimental conditions — disguise the fact, which specially was our object to demonstrate, viz. that there are differences of hereditary nature between the various specimens.

This proof is of considerable interest for the question of fishraces and supports in a high degree the theory previously put forward by me.

January 1919.

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LA VALEUR DE L'INDIVIDU A TITRE DE GÉNÉRATEUR

APPRÉCIÉE SUIVANT LA MÉTHODE DU CROISEMENT DIALLÈLE

PAR

JOHS. SCHMIDT.

I. Caractères qualitatifs et quantitatifs. — Valeur individuelle et valeur générative.

Dans la science génétique, on distingue entre caractères qualitatifs et quantitatifs. A titre d'exemple, on peut citer que la couleur des cheveux d'un homme est du nombre des caractères qualitatifs, tandis que sa taille — évaluée, par exemple, en centimètres — est un caractère quantitatif. Ces derniers se caractérisent par ce fait, que les différences existant entre les individus peuvent s'exprimer comme des différences de degré d'une même unité. On peut mesurer la taille de deux hommes et constater que l'un d'eux est de 10 centimètres plus haut que l'autre. Au contraire, on n'aurait guère chance d'être compris en disant que tel homme aux cheveux noirs les a à tel degré plus noirs que ceux d'un homme roux, par exemple.

Il est malaisé de décider si l'une de ces deux catégories de caractères est plus importante que l'autre. Toujours est-il que presque tout ce qui se rapporte à la productivité d'un individu est susceptible d'être rangé dans celle des caractères quantitatifs. C'est ainsi que la force musculaire des bras d'un homme, la lactation d'une vache, la teneur en sucre d'une betterave, sont des exemples de caractères quantitatifs. Il faut dire que, comparativement à ce que nous savons déjà au sujet de la transmission héréditaire des propriétés qualitatives, les données relatives à l'hérédité des caractères quantitatifs sont très restreintes, à tel point que, dans

une conférence tenue il y a quelques années (1914), le célèbre savant anglais W. BATESON a pu s'exprimer ainsi: «Of the descent of quantitative characters we still know practically nothing» («Au sujet de la transmission de caractères quantitatifs, nous ne savons encore que très peu de chose, presque rien»). A quoi cela tient-il? Évidemment, on ne saurait contester l'importance que présente au point de vue pratique la connaissance de l'hérédité des propriétés quantitatives; tout au contraire: chacun sait que dans tous les pays on fait des efforts persévérants pour «améliorer», «perfectionner» les animaux domestiques et les plantes cultivées. On a aussi entendu dire que ces efforts comportent de grandes difficultés et que dans une large mesure on travaille au hasard. Ceci tient surtout à ce fait, que la valeur purement personnelle d'un individu quelconque ne permet point de conclure à la valeur qu'il possède à titre de générateur, sa valeur générative. Bien des hommes pratiques en savent dire quelque chose, et c'est en réalité la même idée qui s'exprime parfois par des propos tels que: «C'est étrange que des parents aussi excellents puissent avoir des enfants si mauvais!« Ce qui, traduit dans le langage dont nous ferons usage dans ce qui suit, veut dire que la valeur personnelle, «individuelle», des parents est supérieure à leur valeur générative.

La question qui nous intéresse tout d'abord ici, c'est celle-ci: Y a-t-il moyen d'évaluer la valeur générative d'un individu quelconque?

Avant de pouvoir répondre à cette question, il convient de faire quelques remarques préliminaires. La raison principale pour laquelle nous savons si peu de chose de la transmission héréditaire des caractères quantitatifs, réside simplement dans le fait que leur étude comporte des difficultés tout extraordinaires, soit d'ordre théorique soit d'ordre purement pratique. Ce n'est pas ici le lieu d'aborder dans les détails cette question: qu'il suffise de mentionner que les caractères quantitatifs sont dans une large mesure sous la dépendance des conditions extérieures dans lesquelles s'accomplit le développement de l'individu, que ce soit au dedans ou au dehors du corps de la mère. Par suite de ce fait, il est naturellement très souvent difficile de se rendre compte si telle ou telle propriété est héréditaire, ou si elle doit son existence à des conditions externes plus ou moins accidentelles.

II. Détermination de la valeur générative chez les plantes autogamiques. — La Méthode des Lignes Pures.

Au point de vue de l'importance qu'ont les conditions extérieures pour les caractères quantitatifs, aucunes recherches génétiques n'ont apporté plus de lumière que celles dues au botaniste danois W. JOHANNSEN, dont les études sur les «Lignes Pures» furent publiées en 1903. En faisant porter ses recherches sur des plantes absolument autogamiques, telles que les haricots et l'orge, JOHANNSEN put réduire au minimum les difficultés d'ordre théorique qui tiennent à ce que les propriétés d'un individu lui ont été en général transmises de deux côtés différents (par le père et par le mère). Chez les végétaux autogamiques, nous rencontrons, en effet, cette particularité que les parents de chaque individu ne sont pas au nombre de deux, mais qu'il n'y en a qu'un, puisque le mâle et la femelle produisant des gamètes se trouvent réunis en un seul et même individu. En conséquence, dans la conception de JOHANNSEN, une «Ligne Pure» est constituée par l'ensemble des individus qui procèdent d'un seul individu homozygote, absolument autogamique. Parmi les caractères quantitatifs dont l'hérédité a fait l'objet des études de JOHANNSEN, citons à titre d'exemple le poids de la graine d'une certaine espèce de haricot. Partant du mélange dont se compose la récolte provenant d'un champ de haricots ordinaire, il put, au moyen d'appréciations de descendance faites d'après le «Principe des Lignes Pures», isoler des types qui, cultivés à travers plusieurs générations, accusèrent constamment des différences de poids. Il apparut ainsi d'une façon frappante que les caractères individuels — ici, le poids d'une graine — dépendent dans une forte mesure des conditions extérieures qui lui sont offertes au cours de son évolution. Deux haricots d'une même Ligne Pure et qui conséquemment se rattachent, selon le terme de JOHANNSEN, à un même «génotype», peuvent ainsi différer beaucoup de poids; autrement dit, ils peuvent être «phénotypiquement» très différents. Inversement, deux haricots de poids identique et qui partant sont d'un même «phénotype», peuvent fort bien être «génotypiquement» différents, appartenant à deux Lignes Pures qui, cultivées à travers un certain nombre de générations, se montrent différentes de façon constante.

De ce que nous venons de dire, il résulte que la méthode

des «Lignes Pures» de JOHANNSEN nous fournit le moyen de déterminer la valeur générative d'un nombre donné d'individus.

Supposons qu'il s'agisse de découvrir, parmi cent graines de haricots qu'on aurait achetées, quelle est celle qui appartient à la race ayant les graines les plus grosses, le but qu'on se propose étant de produire des haricots présentant le maximum de poids de graine. Pour résoudre ce problème, il ne suffit pas de peser ou mesurer les cent haricots; car ces opérations n'en font connaître que la valeur individuelle. Afin d'en trouver la valeur générative, il est nécessaire de semer les graines, puis de les laisser croître dans des conditions aussi uniformes que possible et, enfin, de récolter les graines de chaque plante séparément. Après la récolte, on pèse les cent lots de haricots un à un, et l'on divise le poids de chaque lot par le nombre de graines qui le composent. Le poids moyen des graines de chaque plante fournirait alors une expression de la valeur de la plante sous le rapport du poids de graine. Pour utiliser pleinement le résultat dans la direction dont il s'agit, on devra donc s'astreindre à la production exclusive de la plante dont la valeur générative se sera trouvée être la plus élevée.

III. Détermination de la valeur générative chez les animaux et plantes allogamiques. — La Méthode du Croisement diallèle.

A. Exposition théorique.

Nous allons maintenant voir comment les choses se passent chez les êtres allogamiques, ainsi que le sont presque tous les animaux et un très grand nombre de plantes. Dans ce cas-ci, la méthode des Lignes Pures n'est évidemment pas applicable, puisqu'elle est assujettie à cette condition que l'espèce considérée soit autogamique. Il est évident cependant que, pour ce qui regarde les organismes allogamiques, il est tout aussi important de savoir déterminer avec certitude la valeur générative d'un être organisé par rapport à sa valeur individuelle. Rappelons seulement, à l'appui de cette thèse, l'intérêt que pour l'éleveur du bétail il y a à travailler avec de bons animaux reproducteurs, autrement dit, des animaux possédant une grande valeur générative.

Dès que nous abandonnons le système si clair et si simple

des Lignes Pures, nous nous heurtons immédiatement à cette complication que, en dehors des influences perturbatrices exercées par les conditions extérieures, chaque individu en particulier a reçu de deux côtés différents, savoir du père et de la mère, la base héréditaire de ses propriétés. Ainsi que JOHANNSEN l'a fait ressortir si souvent dans ses écrits, le grand avantage qu'offre la méthode des Lignes Pures réside précisément dans ce fait qu'elle ne comporte point cette complication[1]). En tout cas, lorsqu'il s'agit de déterminer chez un animal ou une plante allogamiques la valeur générative d'un caractère quantitatif quelconque, il est évident que la tâche se complique singulièrement. Nous avons vu que chez les végétaux autogamiques on détermine la valeur générative comme la moyenne de celle de la descendance. Or, il va de soi que cela n'est pas possible ici, attendu que la descendance d'une femelle quelconque par ex. comprend non seulement les éléments héréditaires provenant de celle-ci, mais aussi ceux qui par la fécondation ont été apportés par le mâle. Il est clair que, pour parvenir à déterminer la valeur générative de la femelle en question, on sera obligé de suivre quelque chemin indirect.

Ayant recours aux données bibliographiques, nous constatons de suite que, si les recherches sur l'hérédité des caractères quantitatifs des êtres organisés ne manquent point — elles sont au contraire nombreuses tant pour les animaux que pour les plantes —, on est loin d'être d'accord sur l'interprétation des résultats. Abstraction faite des cas exceptionnels, et nous en tenant à ce qui paraît être la règle, nous pouvons résumer les résultats en deux énoncés: 1° Le père et la mère semblent généralement exercer une égale influence sur les propriétés héréditaires de la descendance. 2° La descendance d'un père et d'une mère qui diffèrent l'un de l'autre sous le rapport de quelque caractère quantitatif, se trouvera, en moyenne, au voisinage de la moyenne des parents, généralement sans coïncider exactement avec cette dernière[2]).

[1]) JOHANNSEN dit quelque part à ce sujet (1905): «Dans les populations formées par des fécondateurs allogamiques, les choses sont naturellement bien plus compliquées, par suite des croisements ayant lieu continuellement, ... dans les populations allogamiques, on n'a jamais de point d'appui permettant d'établir des types quantitativement différents; ici, tout se confond de façon à paraître inextricable.»

[2]) Cela résulte d'un grand nombre de recherches. Qu'il suffise de mentionner

Ainsi donc, il ne semble pas y avoir accord parfait entre les valeurs moyennes des descendants et des parents. Aussi bien, n'est-on pas, ce me semble, en droit d'attendre un tel accord, vu surtout qu'on n'a pas pu calculer la moyenne des parents à partir de leurs valeurs génératives, mais seulement des valeurs individuelles directement observées. Eu égard à cette circonstance, il paraît légitime d'admettre que la valeur moyenne de la descendance coïnciderait, en réalité, avec la moyenne des valeurs des parents, si le développement de ces derniers avait eu lieu dans les mêmes conditions extérieures que celui de la descendance. Le fait que les deux moyennes ne s'accordent généralement pas tout à fait trouve alors son explication dans cette circonstance que, le plus souvent, les parents et leur postérité n'ont pas eu les mêmes conditions externes pendant leur développement. Tout ce qu'ont appris les études sur les Lignes Pures porte à croire qu'il en est ainsi.

A supposer que les considérations qui précèdent soient correctes, il apparaît que le résultat du croisement d'un mâle et d'une femelle différents au point de vue de quelque propriété quantitative pourra s'exprimer par l'équation:

$$\frac{x + a}{2} = \text{descendance},$$

où x répresente la valeur générative du père, a celle de la mère, tandis que le mot «descendance» dénote la valeur moyenne des descendants. Cette dernière est déterminée par un examen des descendants. Nous avons ainsi une équation où il entre deux inconnues, x et a. Pour pouvoir avancer, il faut procurer encore une égalité contenant l'une de ces deux inconnues. A cet effet, nous pouvons, par ex., faire accoupler la même femelle a avec

un exemple tiré de celles du chercheur américain W. E. Castle (cité d'après un compte rendu d'Arnold Lang dans la Zeitschrift für induktive Abstammungs- und Vererbungslehre, IV, 1911). Une lapine aux oreilles très longues (longueur des oreilles: 225 millimètres) fut croisée avec un lapin à oreilles courtes (105 mm). Les huits petits présentaient une longueur d'oreilles moyenne de 146mm,4, voisine de la moyenne des oreilles de leurs parents. Procédant ensuite à un croisement inverse, «réciproque», on employa comme femelle un individu appartenant à la race aux oreilles courtes, comme mâle un individu de celle aux oreilles longues, la longueur des oreilles étant respectivement de 100 et de 210 mm. Celle des six petits s'élevait en moyenne à 148mm,8 (moyenne des parents: 155 mm).

un autre mâle, y, pour déterminer la moyenne de la lignée nouvelle. Nous avons alors:

$^1/_2 (x + a)$ = descendance 1 et $^1/_2 (y + a)$ = descendance 2,

d'où par soustraction:

$$^1/_2 (x - y) = \text{descendance 1} - \text{descendance 2}.$$

Nous sommes donc ainsi arrivés à déterminer la différence entre les valeurs génératives des deux mâles x et y.

De la même manière nous pouvons déterminer la différence entre deux femelles a et b, en les faisant accoupler avec un seul et même mâle et en examinant ensuite les descendants. On aura alors:

$^1/_2 (x + a)$ = descendance 1 et $^1/_2 (x + b)$ = descendance 2,

d'où: $^1/_2 (a - b)$ = descendance 1 — descendance 2.

On sera ainsi arrivé à déterminer la différence entre les deux femelles a et b, c'est-à-dire, à les classer selon leur valeur générative.

En procédant d'une manière analogue à celle que nous venons d'esquisser, on peut, d'une façon générale, croiser A mâles et B femelles. Il se produira par là A × B différentes combinaisons de descendance, auxquelles il faut, bien entendu, avoir soin de fournir pour leur développement des conditions extérieures aussi uniformes que possible. Par soustraction des équations obtenues, ainsi que nous l'avons indiqué ci-dessus, on peut déterminer la différence entre les femelles, d'une part, et, de l'autre, entre les mâles. De cette façon, on parviendra à classer d'après la grandeur de leurs valeurs génératives toutes les femelles, d'un côté, et, de l'autre, tous les mâles. Nous voilà ainsi parvenus à l'égard des espèces allogamiques à un résultat analogue à celui obtenu pour les végétaux autogamiques à l'aide de la méthode des Lignes Pures.

La méthode que nous venons d'exposer se caractérise particulièrement par les croisements répétés, par l'élevage de la progéniture dans des conditions identiques autant que possible, par la détermination des valeurs moyennes des descendants et, finalement, par un calcul peu compliqué.

J'appellerai ce procédé la Méthode du Croisement diallèle[1])

[1]) de l'adjectif grec *διάλληλος* (comp. par-allèle), qui correspond de très près à l'allemand «*durcheinander*», ainsi que me l'a fait observer M. le Professeur J. L. Heiberg. En anglais, j'appelle la méthode «the Method of Diallel

ou de l'Entrecroisement complet, étant donné que ce qui le caractérise principalement, ce sont les entrecroisements complets de tous les individus en question, pour ainsi dire, «*durcheinander*».

Il y a lieu de remarquer ici que, en ce qui concerne l'exactitude des considérations qui nous ont conduit à formuler cette méthode, il nous est possible de la soumettre à une vérification par laquelle les valeurs numériques résultant de nos calculs se trouveront vérifiées en même temps.

Si nous complétons les croisements sus-indiqués des mâles x et y avec une femelle b, nous aurons les quatre équations suivantes:

$^1/_2 (x + a)$ = descendance 1, $\quad$ $^1/_2 (x + b)$ = descendance 3,
$^1/_2 (y + a)$ = descendance 2, $\quad$ $^1/_2 (y + b)$ = descendance 4.

En défalquant l'équation 2 de 1 et l'équation 4 de 3, on aura:

$^1/_2 (x - y)$ = descendance 1 — descendance 2
$^1/_2 (x - y)$ = descendance 3 — descendance 4.

Il s'agit donc de vérifier si la descendance 1 — descendance 2 est égale à descendance 3 — descendance 4, vérification qui peut s'effectuer au moyen d'un examen des échantillons de descendance.

Si la vérification — qui peut, cela va sans dire, s'opérer aussi en soustrayant l'égalité 3 de 1 et 4 de 2 — donne dans l'un et l'autre cas le même résultat pour $^1/_2 (x - y)$ (ou pour $^1/_2 (a - b)$), il est démontré par là que nos considérations sont bien exactes et que nous sommes ainsi à même de déterminer, par la méthode du Croisement diallèle, la différence entre les valeurs génératives des deux individus du même sexe, respectivement $x - y$ et $a - b$.

Dans le cas considéré, le croisement diallèle n'était effectué qu'avec deux mâles (x et y) et deux femelles (a et b). Il va sans dire cependant, qu'on peut fort bien exercer le contrôle, même si l'essai porte sur un beaucoup plus grand nombre d'individus, soit par ex. cinq mâles et cinq femelles. Il devient même alors d'autant plus effectif, puisqu'on n'aura pas moins de cinq déterminations de chaque différence; $a - b$ sera déterminé non pas deux, mais cinq fois, et ainsi de suite. De cette façon, non seulement on obtiendra un contrôle plus complet, mais les déterminations seront plus sûres. Ainsi, quand on a déterminé cinq

Crossing» ou «Method of Complete Intercrossing», cette dernière désignation étant due à Mlle K. Smith, D. Sc., qui a bien voulu se charger des calculs mathématiques auxquels le présent mémoire a donné lieu.

fois $a - b$ et $x - y$, il sera raisonnable d'opérer sur la moyenne des cinq déterminations. On parvient ainsi à réduire considérablement l'inexactitude due à l'impossibilité de satisfaire tout à fait à la condition théorique sans laquelle notre méthode n'est plus applicable, à savoir que tous les descendants expérimentés doivent s'être développés dans des milieux rigoureusement identiques sous tous les rapports.

Si dans tous les cas il est nécessaire d'avoir soin de procurer à tous les échantillons de descendance des conditions d'existence pareilles autant que possible, nous pouvons dire dès maintenant que, tant qu'il ne s'agit que d'établir les valeurs génératives des individus considérés, il importe beaucoup moins que ces conditions soient exactement les mêmes que celles dans lesquelles les parents se sont développés. Nous reviendrons sur cette question plus loin, lors de la description de mes expériences sur ce sujet, et je me bornerai ici à mentionner que dans la pratique il conviendra de s'arranger de manière à élever aussi bien les parents que leurs descendants dans des conditions sensiblement identiques et normales.

C'est ici le lieu de préciser au moyen d'un exemple les avantages que nous avons obtenus en étant à même de déterminer la différence entre les valeurs génératives de deux ou plusieurs individus au point de vue d'une propriété quantitative quelconque. Supposons que pour un éleveur de porcs il s'agisse de leur faire produire une progéniture à croissance rapide. Or, le temps qu'un petit cochon met à atteindre un certain poids dépend naturellement des soins qu'on lui donne, de la manière dont on le nourrit; en d'autres termes, il est influencé dans une forte mesure par les conditions extérieures au sens le plus large du mot. Cependant, ce fait ne change en rien cet autre fait, que deux populations, soit de porcs ou d'autres animaux quelconques, même quand elles sont entretenues de la même manière, n'en peuvent pas moins se développer avec une vitesse très inégale par suite d'une origine différente. Je tiens à insister sur ce fait parce que d'aucuns paraissent avoir de la difficulté à faire une distinction bien nette entre ces deux choses: croissance rapide due à un entretien soigné, d'un côté, et, de l'autre, croissance rapide due à des dispositions héréditaires. Il est évident que — abstraction faite de la question d'entretien — le possesseur de bestiaux doués de la propriété de croître rapidement a, par cela

même, un avantage indubitable sur celui dont les bestiaux sont lents à se développer.

Dans l'exemple choisi, il s'agit donc de sélectionner, en vue de la reproduction, entre les porcs ou bestiaux qu'on possède, ceux qui, toutes les conditions d'ailleurs égales, présentent la propriété d'engendrer des petits capables d'atteindre dans le minimum de temps un certain poids, soit A kilos. Une pareille lignée à minimum de «temps de développement» permettra d'utiliser de la façon la plus économique à la fois le temps, l'espace et le fourrage.

Supposons que l'éleveur de porcs possède deux jeunes verrats: x et y, et qu'il tienne à savoir lequel est le meilleur procréateur au point de vue de la production d'une descendance à croissance rapide. Dans le langage employé ici, la question se pose donc ainsi: Lequel des deux verrats, de x ou de y, présente la plus grande valeur générative? et quelle est la différence, s'il y en a?

Supposons encore que les deux verrats soient apparemment équivalents, l'un et l'autre ayant mis le même temps, soit quatre-vingt-six jours, à atteindre le poids de A kilos. La différence entre les valeurs individuelles est donc égale à zéro.

Employons maintenant les deux verrats, x et y, à une expérience pratiquée suivant la méthode du croisement diallèle, en accouplant l'un et l'autre avec deux truies, a et b. Examinons ensuite combien de jours les petits engendrés mettront à atteindre le poids de A kilos, et calculons pour chaque portée la moyenne du «temps de développement» des petits cochons. Admettons que nous trouvions:

$$\begin{array}{llll} & {}^1/_2\,(x+a) = 90 & {}^1/_2\,(x+b) = 93 \\ & {}^1/_2\,(y+a) = 83 & {}^1/_2\,(y+b) = 86, \\ \text{d'où} & x - y = 14 \text{ et} & b - a = 6. \end{array}$$

Ainsi, cette expérience nous aura montré que, des deux verrats x et y, qui avaient l'un et l'autre mis quatre-vingt-six jours a atteindre le poids de A kilos, celui désigné par y est sensiblement supérieur comme procréateur; plus exactement, y est de quatorze jours meilleur, si l'on peut s'exprimer ainsi. En fait, nous avons trouvé que y est égal à $x - 14$, ce qui veut dire que le verrat y communique à sa progéniture la propriété d'atteindre le poids de A kilos dans un temps qui est de 14

jours plus court que celui mis par la progéniture de *x* pour atteindre le même pôids.

De la même manière nous avons trouvé que les valeurs génératives des deux truies *a* et *b* présentaient une différence de six jours en faveur de *a*, cette dernière valeur s'étant trouvée égale à *b* — 6.

Une pareille appréciation des valeurs génératives des deux verrats (et truies) aura naturellement pour conséquence qu'on ne fera servir à la reproduction que le verrat *y* et la truie *a*. On obtiendra de la sorte une population de porcs ayant, en moyenne, un «temps de développement» de quatre-vingt-trois jours, c'est-à-dire qui ne mettront en moyenne que 83 jours à atteindre le poids de A kilos. Si l'on avait choisi l'autre verrat, *x* — qui semblait également bon —, la truie *a* aurait donné une population dont les individus mettraient en moyenne 90 jours à atteindre le poids de A kilos, tandis que la postérité de *b* n'aurait atteint ce poids qu'au bout de 93 jours.

Le but de l'exemple qui précède, et que nous avons choisi arbitrairement, est simplement de mettre en évidence les avantages qu'on pourrait réaliser en faisant l'appliquation pratique de la méthode du croisement diallèle. Bien entendu, nous eussions pu tout aussi bien choisir chez le porc une autre propriété quantitative d'importance économique, de même qu'on aurait pu faire servir d'exemple un autre animal domestique ou bien une plante cultivée quelconque. Ce que nous avions en vue était uniquement d'illustrer par un exemple la manière d'agir qu'il conviendrait d'employer.

B. Contrôle expérimental.

D'après l'exposé qui précède, il n'y aurait donc rien qui empêche de déterminer, au moyen de la méthode du croisement diallèle, la valeur générative des animaux et des plantes allogamiques et d'obtenir par là des résultats importants au choix des individus propres à reproduire la race.

La question se pose maintenant de savoir comment les choses se passeront lorsqu'il s'agira de faire l'application pratique de la méthode. Et c'est là ce qui importe avant tout; car toute cette affaire ne vaudrait évidemment pas grand'chose si la théorie ne s'appuyait sur des données expérimentales.

Dans l'introduction à ce mémoire, nous avons vu que l'étude de la transmission héréditaire des propriétés quantitatives présente des difficultés toutes particulières, dues surtout aux influences perturbatrices exercées par le milieu. Quand on aura à faire la preuve de la théorie de notre méthode, il sera donc de la dernière importance d'avoir à sa disposition des éléments bien appropriés. Ce dont il s'agit principalement dans l'épreuve de cette théorie, c'est d'arriver à des moyennes bien exactes et comparables. Il y a ici deux points qui doivent surtout entrer en ligne de compte: Il faut tout d'abord choisir une espèce à descendance aussi nombreuse que possible, et puis on doit pouvoir maîtriser en quelque sorte l'influence des conditions extérieures. Lorsqu'il s'agit d'une vérification rigoureuse de la base théorique de la méthode, la première de ces deux exigences rend inapplicables certains animaux supérieurs peu féconds, tels que le cheval et la vache, alors que la seconde exigence tend à empêcher notamment l'emploi d'un bon nombre de plantes. Il sera cependant absolument indispensable de satisfaire à ces deux exigences; sans cela, il pourra arriver que des circonstances purement accidentelles (par ex., des observations trop peu nombreuses) ou bien des inexactitudes (des observations discordantes) empêchent de mettre en évidence les lois auxquelles les phénomènes observés sont constamment soumis, lors même qu'elles nous échappent à cause des difficultés techniques.

Or, je crois avoir trouvé dans diverses espèces de poissons des objets se prêtant éminemment aux expériences tentées en vue de la vérification de la théorie qui nous occupe[1]).

Des études poursuivies depuis bien des années sur les races de poissons m'avaient appris que les poissons offrent des caractères quantitatifs qui se prêtent particulièrement bien à des investigations exactes. Parmi ces caractères, on peut citer le nombre de différents organes, tels que vertèbres, rayons des nageoires, etc. En effet, d'abord ces organes ou parties d'organe sont faciles à compter avec une certitude absolue, sans qu'on ait besoin de recourir à une appréciation personnelle; et puis ils sont passablement variables en nombre d'un individu à l'autre, bien que, d'un autre côté — et ceci constitue un avan

[1]) Il n'est pas besoin, je pense, de faire observer que la marche de ces études a suivi le sens inverse: les expériences ont été réalisées d'abord, et c'est d'elles qu'est résultée ensuite la théorie.

tage de plus — ils ne soient pas assujettis à l'influence des conditions externes au même degré vraiment excessif que le sont tant d'autres caractères quantitatifs chez les animaux et bien plus encore chez les plantes. Ajoutez encore à tout cela que les poissons présentent le grand avantage d'être très prolifiques: la descendance se compte ici par milliers.

Après plusieurs essais infructueux faits avec certains poissons des tropiques élevés dans des aquariums, il me vint à l'idée que la truite commune (*Salmo trutta* L.) devait être propre à servir d'objet de ces recherches. Dans un grand nombre d'établissements d'éclosion artificielle dans mon pays, plus particulièrement dans le sud de la Jutlande, on réalise tous les ans une fécondation artificielle d'œufs de truite, qu'on met ensuite en vente à l'état d'œufs («Øjen-Æg») ou à des états ultérieurs de développement. En somme, on a créé dans ce domaine une technique parfaitement élaborée dans tous les détails, fait dont nous pouvions tirer profit pour des études génétiques comme celles que nous avions en vue. Grâce à l'obligeante intervention de M. P. JESPERSEN, maître-ès-sciences, j'eus alors la bonne fortune d'être mis en rapport avec un homme pratique qui s'est obligeamment chargé d'effectuer les expériences projetées. Je suis heureux de présenter à cet homme: M. le Directeur A. CHRISTIANSEN, à Jested Mølle, près de Kongeaaen (en Jutlande), l'hommage de ma gratitude. En fait, sans le vif intérêt qu'il a apporté à ces recherches, sans l'exactitude consciencieuse dont il a fait preuve et qui est de rigueur pour que leurs résultats ne soient pas entièrement faussés, il m'eût été impossible de mener à bonne fin les investigations entreprises dans ce domaine resté inexploré jusqu'ici. Je dois aussi de la reconnaissance à mes aides naturalistes, MM. le Dr Ø. WINGE et VILH. EGE, maître-ès-sciences, pour le concours précieux qu'ils n'ont cessé de m'apporter.

Les premières expériences furent instituées en 1915, et une série d'autres les ont suivies, toujours sous la direction de M. CHRISTIANSEN, Jested Mølle. En outre de ces recherches pratiquées en Jutlande, nous avons procédé, dans notre Laboratoire Carlsberg, à des expériences supplémentaires ayant principalement pour but de rechercher quelle est l'influence qu'exercent les conditions extérieures sur les caractères quantitatifs de la truite sur lesquels ont porté nos études génétiques.

La manière dont s'opère la fécondation artificielle des œufs

de truites consiste à exprimer des poissons parents les œufs et le sperme, puis à mélanger ceux-ci en les remuant. Les œufs ainsi fécondés sont laissés en repos dans des bacs traversés par un courant d'eau. L'éclosion se fait ensuite dans le courant de quelques mois. L'alevin éclos s'alimente, dans les premiers temps, du contenu du grand sac vitellin, et c'est seulement lorsque celui-ci aura été absorbé que le petit poisson commence à se nourrir par la voie buccale. Les individus examinés par moi furent sacrifiés au moment de leur développement où ils avaient achevé de consommer le contenu du sac vitellin. A ce moment, en effet, tous les caractères que nous nous étions proposé d'étudier sur les petits poissons mesurant environ $2^{cm},5$, étaient tout à fait développés. Nous nous sommes appliqué à constater, entre autres, le nombre de vertèbres et la longueur totale du petit poisson au moment de la disparition du sac vitellin, deux caractères qui, au point de vue dont il s'agit, offrent cet avantage que toutes les perturbations qui pourraient résulter de ce que les différents individus en voie de développement prennent des quantités inégales d'aliment se trouvent exclues, attendu que les animaux sont tués avant l'époque où ils commencent à chercher de la nourriture de dehors.

Je vais relater ici deux expériences pratiquées suivant la méthode du croisement diallèle, l'un en 1915, l'autre en 1917 et 1918, toutes les deux par M. CHRISTIANSEN à Jested Mølle. La première de ces expériences porte sur l'hérédité du nombre des vertèbres, la seconde sur celle de la longueur totale au moment de la disparition du sac vitellin. En faisant l'exposé de ces expériences, nous avons principalement en vue de vérifier l'exactitude des considérations théoriques énoncées au chapitre précédent et, par là, de réaliser un contrôle de la méthode du croisement diallèle. J'ai cru devoir partager cet exposé en deux parties, **a** et **b**, dont la première a simplement pour but de montrer comme ladite méthode peut servir à classer un certain nombre d'individus selon leur valeur générative, tout en fournissant un contrôle de l'exactitude de ce classement, tandis que dans la seconde partie nous nous attacherons à traiter à fond la question de savoir comment dans un cas donné on peut s'arranger de manière à obtenir une expression des valeurs génératives en comparaison des valeurs personnelles des mêmes individus. Pour ceux de mes lecteurs qui ne s'intéressent qu'au principe même de la méthode, l'exposé fait dans la première partie suffira.

D'ailleurs, je compte publier plus tard une description plus détaillée des expériences en question, ainsi que de la base théorique de la méthode.

a. Classification d'un certain nombre de mâles ou de femelles selon leurs valeurs génératives.

Expérience N° 1. Caractère: Nombre des vertèbres.

Durée de l'expérience: du 15 janvier 1915 au 4 juin 1915.

On a effectué un croisement diallèle avec six truites, savoir deux mâles et quatre femelles. Voici la liste des animaux d'expérimentation avec leurs caractères concernés:

	Mâles	Longueur (en cm)	Poids (grammes)	Vertèbres (valeurs individuelles)
	x	40	700	**58**
	y	56	2000	**60**
	Femelles			
	a	44	830	**59**
	b	37	490	**60**
	c	39	635	**58**
	d	62	1970	**58**

Le croisement diallèle donna naissance à 8 (2 × 4) différentes combinaisons de lignée, désignées dans la suite par les lettres indiquant les parents.

Le tableau ci-après montre le nombre moyen des vertèbres des descendants, déterminé après numération des vertèbres dans un grand nombre d'exemplaires de chaque échantillon[1]).

Nombre moyen de vertèbres dans la descendance.

(Pères: *x*, *y*; mères: *a*, *b*, *c*, *d*).

Combinaison . . .	*xa*	*xb*	*xc*	*xd*
	58.019	**58.488**	**57.370**	**57.729**
Combinaison . . .	*ya*	*yb*	*yc*	*yd*
	58.900	**59.346**	**58.140**	**58.457**

[1]) Chaque échantillon comprenait un nombre considérable d'individus, à savoir 260, 215, 257, 323, 467, 276, 43, 416. Cette circonstance jointe au fait que les limites de variation sont assez étroites (2—4), a permis de déterminer les moyennes avec un haut degré de précision.

Les nombres consignés dans ce tableau sont disposés de telle sorte qu'ils se répartissent en deux séries horizontales formées chacune par quatre combinaisons de descendance, et en quatre colonnes verticales constituées chacune par deux combinaisons. Les nombres formant les deux séries horizontales se rapportent aux échantillons qui ont le même père (respectivement x et y), ceux des quatre colonnes aux échantillons ayant la même mère (respectivement a, b, c, d). En comparant entre eux les nombres des séries horizontales, on peut ainsi classer les valeurs génératives des femelles d'après le nombre moyen de vertèbres dans la descendance. Nous trouvons ainsi pour la série supérieure (celle où le mâle x est le père) la succession suivante, disposée par ordre de grandeur décroissante:

$$b \text{ — } a \text{ — } d \text{ — } c,$$

et pour la série inférieure (père: mâle y):

$$b \text{ — } a \text{ — } d \text{ — } c.$$

Nous sommes ainsi parvenus à classer les quatre femelles d'après la grandeur de la valeur générative, et ayant en même temps démontré que les membres des deux séries se succèdent de façon identique, nous sommes arrivés à faire vérification de l'exactitude du principe suivant lequel nous les avons classés.

D'une manière analogue nous pouvons classer les deux mâles x et y en faisant la comparaison des séries verticales. Nous aurons alors, en plaçant en haut la valeur la plus élevée:

Série 1	Série 2	Série 3	Série 4
y	y	y	y
x	x	x	x

Ainsi donc, le classement donne aussi des résultats concordants pour les mâles. En conséquence, il résulte de notre expérience qu'à l'aide de la méthode du croisement diallèle nous avons pu établir que, des deux mâles x et y, c'est y qui avait la plus grande valeur générative, tandis que les femelles se suivaient ainsi: b — a — d — c.

Expérience N° 2. Caractère: Longueur totale, en millimètres, au moment de la disparition du sac vitellin.

Durée de l'expérience: du 29 novembre 1917 au 8-9 mai 1918.

On a réalisé un croisement diallèle avec huit truites: quatre mâles et quatre femelles; cependant, il n'a produit que douze échantillons de descendants utilisables. Dans chaque échantillon, on a mesuré la longueur de cinquante exemplaires. Les moyennes se trouvent inscrites sur le tableau suivant, où x, y, z et v désignent les pères, a, b et c, les mères.

Longueur moyenne de la descendance, en millimètres.

(Pères: x, y, z, v; mères: a, b, c).

Combinaison	ax	ay	az	av
	23.06	**23.64**	**22.38**	**23.32**
Combinaison	bx	by	bz	bv
	24.20	**24.76**	**24.10**	**24.70**
Combinaison	cx	cy	cz	cv
	23.50	**24.38**	**23.36**	**23.82**

Dans les séries horizontales nous avons des échantillons nés de la même mère, tandis que ceux des colonnes verticales ont le même père. La comparaison des nombres des séries horizontales montre que les valeurs génératives des quatre mâles se succèdent comme suit:

Série 1	Série 2	Série 3
y	y	y
v	v	v
x	x	x
z	z	z

De la même manière, en comparant les nombres des séries verticales, on peut trouver l'ordre décroissant des valeurs génératives des femelles, et l'on arrive ainsi au tableau qui suit:

Série 1	Série 2	Série 3	Série 4
b	b	b	b
c	c	c	c
a	a	a	a

De même que dans l'expérience précédente sur le nombre des vertèbres, notre classement donne donc encore ici des résultats concordants dans tous les cas, aussi bien pour les mâles que pour les femelles[1]). Il est évidemment hors de doute que l'accord constaté dans l'ordre de succession n'est point un pur hasard. En ce qui concerne la dernière expérience, où le même ordre a été observé trois fois pour les quatre mâles et quatre fois pour les trois femelles, un pareil hasard ne se produirait qu'une seule fois sur 7200 si l'accord précité était de nature purement accidentelle.

Ainsi donc, les deux expériences que nous venons de relater et dont l'une concerne un caractère qui se compte (le nombre des vertèbres), l'autre un caractère qui se mesure (la longueur), ont donné des résultats concordants. Dès lors, nous estimons avoir démontré de façon suffisante que la méthode du croisement diallèle est bien applicable à la classification des valeurs génératives d'un certain nombre d'individus.

b. Exemple de la détermination de la valeur générative d'un certain nombre d'individus.

Il convient de choisir à titre d'exemple l'expérience N° 1 concernant le nombre des vertèbres, parce qu'elle nous permet de déterminer aussi les valeurs individuelles des animaux d'expérimentation, de telle sorte qu'une comparaison entre celles-ci et les valeurs génératives devient possible, comparaison qui évidemment présente un certain intérêt. Les animaux et leurs valeurs individuelles se trouvent énumérés à la page 15. Ci-après, je donne un aperçu des nombres individuels des parents.

[1]) Il appert donc par cette expérience que la longueur totale du petit poisson est également un caractère héréditaire. En même temps nous pouvons constater, non seulement que le père influe sur la taille de la progéniture, mais encore qu'il est possible de classer à cet égard les quatre mâles. Aussi, le mâle *y* donne avec chacune des femelles des descendants plus longs que ne le fait aucun des trois autres mâles. De prime abord, on aurait été tenté de croire que la longueur des petits poissons était déterminée seulement par la grosseur de l'œuf et, partant, par la quantité de nourriture offerte à l'alevin. — Comme un autre fait assez curieux, on peut remarquer que notre expérience nous a permis d'évaluer la longueur qu'avaient à l'état d'alevin les poissons qui lors de cette expérience avaient atteint l'âge adulte.

Moyennes de vertèbres des couples de parents et de leur progéniture.

(xa etc. désignent la moyenne des vertèbres de la progéniture, $^1/_2\ (x + a)$ etc. celle des vertèbres des parents. Les nombres de la dernière colonne sont égaux à ceux de la première + 0.5).

xa	**58.019**	$^1/_2\ (x + a)$	**58.5**	$xa + 0.5$	**58.519**
xb	**58.488**	$^1/_2\ (x + b)$	**59.0**	$xb + 0.5$	**58.988**
xc	**57.370**	$^1/_2\ (x + c)$	**58.0**	$xc + 0.5$	**57.870**
xd	**57.729**	$^1/_2\ (x + d)$	**58.0**	$xd + 0.5$	**58.229**
ya	**58.900**	$^1/_2\ (y + a)$	**59.5**	$ya + 0.5$	**59.400**
yb	**59.346**	$^1/_2\ (y + b)$	**60.0**	$yb + 0.5$	**59.846**
yc	**58.140**	$^1/_2\ (y + c)$	**59.0**	$yc + 0.5$	**58.640**
yd	**58.457**	$^1/_2\ (y + d)$	**59.0**	$yd + 0.5$	**58.957**

Il ressort de ce tableau que la moyenne des vertèbres de la descendance (première colonne) correspond sensiblement à la moyenne des valeurs individuelles des parents (dernière colonne), surtout en ce qui concerne l'ordre de succession. Cependant, en y regardant de plus près, on s'aperçoit que cela n'est vrai que sous certaines réserves. Tout d'abord, on voit que les moyennes de la descendance sont dans tous les cas inférieures à celles des parents, différence qui en moyenne égale $^1/_2$ vertèbre. Ceci n'a pourtant rien qui puisse étonner, si l'on prend en considération les résultats qu'ont donnés des expériences effectuées à cet égard au Laboratoire Carlsberg. Ceux-ci ont, en effet, montré que le nombre moyen des vertèbres dans les échantillons de descendance ayant le même père et la même mère, mais ayant vécu dans des conditions inégales, pouvait varier de plus de $^4/_5$ vertèbre. Dès lors, il n'est plus étonnant que dans l'expérience actuelle les moyennes de la descendance se trouvent partout inférieures à celles des parents. On peut sans doute s'expliquer ce fait en admettant, ou bien que tous les descendants ont été pendant leur évolution soumis à certaines conditions extérieures qui auraient eu pour effet de diminuer le nombre moyen des vertèbres, ou bien que les valeurs individuelles des parents ont été particulièrement élevées. Dans les circonstances données, il semble cependant légitime d'admettre que les descendants ont tous été sous l'influence de conditions extérieures sensiblement

égales, et dès lors, en vue de rendre plus comparables les nombres de la descendance et ceux des parents, il doit être permis d'ajouter à toutes les moyennes des descendants le même nombre. Or, ayant vu que ces dernières étaient, en moyenne, d'environ $^1/_2$ moins élevées que celles des parents, il semble tout indiqué d'augmenter de $^1/_2$ les moyennes de la descendance. De là résultent les nombres formant la dernière colonne du tableau ci-dessus. En comparant les nombres des deux dernières colonnes, on remarque toujours un certain désaccord entre les moyennes de la descendance et celles des parents. Il est surtout frappant que les deux combinaisons xc et xd (ainsi que yc et yd) accusent, pour ce qui concerne la descendance, des différences notables, quoique le père soit le même dans les deux cas (respectivement x et y) et que les mères présentent le même nombre de vertèbres (les femelles e et d ayant chacune 58 vertèbres). D'où vient ce désaccord? Et pourquoi se traduit-t-il, dans la série x aussi bien que dans la série y, par ce fait que, quoique les poissons c et d aient le même nombre de vertèbres, savoir cinquante-huit, la combinaison contenant d donne à sa descendance un nombre plus élevé que ne fait celle qui contient c?

Si l'on tient compte des résultats obtenus par W. JOHANNSEN dans ses recherches sur les Lignes Pures (comp. p. 3—4), on est porté à croire — et les conditions données paraissent même rendre vraisemblable — que l'explication du désaccord dont il est question doit nécessairement être cherchée dans ce fait que les deux femelles c et d, malgré qu'elles «portent», si je puis m'exprimer ainsi, toutes les deux un nombre de vertèbres de 58, ne sont pas en réalité identiques à cet égard; en d'autres termes, ces deux femelles, tout en présentant la même valeur individuelle, diffèrent l'une de l'autre sous le rapport de leur valeur générative.

En effet, nous ne devons pas perdre de vue qu'elles se sont probablement développées dans des conditions extérieures dissemblables. Il faut bien remarquer que ce sont des poissons «sauvages», provenant vraisemblablement chacun de son «nid» et ayant sans doute atteint des âges différents. En conséquence, ils ont selon toute vraisemblance parcouru les premières phases de leur développement (leur période «sensible») dans des conditions différentes, qui auront eu pour effet d'augmenter chez l'un d'eux le nombre de vertèbres et de le réduire chez l'autre,

tout comme nous avons eu l'occasion de l'observer dans nos expériences de laboratoire.

La question sera ensuite de savoir si l'expérience qui nous occupe nous permet de déterminer le nombre génératif de vertèbres de nos animaux d'expérimentation, autrement dit, leurs valeurs à titre de générateur.

Si nous désignons par x, y, a, b, c, d les valeurs génératives cherchées, nous aurons en utilisant les nombres de la dernière colonne du tableau p. 19:

$\frac{1}{2}(x + a) = \mathbf{58.519}$ $\frac{1}{2}(y + a) = \mathbf{59.400}$, dont $\frac{1}{2}(y - x) = \mathbf{0.881}$
$\frac{1}{2}(x + b) = \mathbf{58.988}$ $\frac{1}{2}(y + b) = \mathbf{59.846}$, » $\frac{1}{2}(y - x) = \mathbf{0.858}$
$\frac{1}{2}(x + c) = \mathbf{57.870}$ $\frac{1}{2}(y + c) = \mathbf{58.640}$, » $\frac{1}{2}(y - x) = \mathbf{0.770}$
$\frac{1}{2}(x + d) = \mathbf{58.229}$ $\frac{1}{2}(y + d) = \mathbf{58.957}$, » $\frac{1}{2}(y - x) = \mathbf{0.728}$

En prenant la moyenne de ces quatre déterminations de $\frac{1}{2}(y - x)$, nous obtiendrons $y - x = 1.1618$.

Ainsi donc, à supposer que les considérations énoncées soient correctes, nous avons pu démontrer que la valeur générative du mâle y est de 1.618 plus élevée que celle du mâle x, quoique la différence entre leurs valeurs individuelles soit plus grande, soit $60 - 58 = 2$.

D'une manière analogue, nous pouvons, en soustrayant l'une de l'autre les équations ci-dessus, déterminer les différences entre les valeurs génératives des femelles et montrer par là qu'elles ne coïncident pas avec les différences des valeurs individuelles.

Cependant, il ne s'agissait pas seulement de déterminer les différences, mais encore d'obtenir une expression des valeurs génératives elles-mêmes. Afin d'y arriver, il faudra avoir soin de rendre comparables les nombres des parents, et à cet effet il sera nécessaire de les rapporter tous aux mêmes conditions extérieures. Moins importe quelles soient ces conditions. Nous pourrons par ex. en posant $x = 58$ (ce qui est la valeur personnelle trouvée pour cet individu) ramener l'ensemble de nos animaux d'expérimentation a des conditions extérieures telles que — pour employer la terminologie de JOHANNSEN — le génotype x serait amené à réaliser 58 vertèbres. Puisque nous avons trouvé $y - x = 1.618$, nous arrivons ainsi à

$$x = 58 \text{ et } y = 59.618.$$

En faisant entrer alors la valeur de x dans la première série

d'équations $[^1/_2 (x + a)]$ etc. et la valeur de y dans la seconde série $[^1/_2 (y + a)]$ etc., nous obtiendrons deux déterminations de la valeur de chaque femelle. Elles se trouvent inscrites sur le tableau ci-après, où la première colonne contient les valeurs génératives calculées à partir de la série x, la dernière celles provenant de la série y.

	Série x	Série y	Moyennes des deux séries
a	59.038	59.182	59.110
b	59.976	60.074	60.025
c	57.740	57.662	57.701
d	58.458	58.296	58.377

La concordance que présentent les nombres de la première et de la deuxième colonne, lesquels ont été déterminés indépendamment les uns des autres, vient confirmer l'exactitude de nos considérations. En pratique, on comptera naturellement avec la moyenne des déterminations relatives à chaque individu, de même que nous avons fait ci-dessus en déterminant $y - x$. Par là on arrive, pour chaque détermination, à réduire toutes les inexactitudes dues à ce fait que la condition théorique à laquelle la méthode est à la rigueur soumise, à savoir que tous les échantillons de descendants devraient s'être developpés dans des circonstances essentiellement identiques, ne saurait guère jamais être remplie en réalité.

Si nous prenons la moyenne des deux valeurs indiquées plus haut pour chacune des quatre femelles, nous arrivons aux nombres de la dernière colonne du tableau. Ceux-ci représentent donc les valeurs génératives qui ont été déterminées à partir de la supposition que la valeur générative du mâle x coïncide avec sa valeur individuelle. C'est là assurément une hypothèse arbitraire et, de fait, nous aurions pu donner à x une autre valeur quelconque, à la seule condition que les autres nombres, auxquels on arriverait par des calculs, seraient plausibles, c'est-à-dire tels qu'on les rencontre réellement pour l'espèce et le caractère concernés. Cependant, il sera logique d'arranger le calcul de telle manière que l'ensemble des écarts que présentent les valeurs génératives par rapport aux valeurs individuelles se réduise au minimum. En agissant de cette façon au lieu de poser $x = 58$, nous arriverons au résultat qui suit:

Valeurs génératives et valeurs individuelles des six animaux d'expérimentation.

Symboles	Valeurs génératives	Valeurs individuelles	Différence
x	**58.099**	**58**	+ 0.099
y	**59.717**	**60**	— 0.283
a	**59.011**	**59**	+ 0.011
b	**59.926**	**60**	— 0.074
c	**57.602**	**58**	— 0.398
d	**58.278**	**58**	+ 0.278

Quoique, en déterminant les valeurs generatives, nous nous soyons arrangés pour nous rapprocher autant que possible des valeurs individuelles, on constate pourtant une différence considérable. Ce sont surtout les deux femelles c et d, ayant l'une et l'autre 58 vertèbres, qui se montrent notablement différentes; la valeur générative de l'une d'elles étant de 0.398 inférieure, celle de l'autre de 0.278 supérieure à 58; dans d'autres expériences, j'ai même trouvé que, dans deux individus personnellement identiques, la valeur générative pouvait différer de $1^1/_2$ vertèbre. Inversement, j'ai observé que deux individus personnellement différents étaient sensiblement identiques sous le rapport de leur valeur générative.

Comme nous l'avons vu, la justesse de nos considérations s'est trouvée vérifiée par la concordance des nombres consignés dans le tableau p. 21. Nous pouvons effectuer le contrôle d'une autre manière en faisant entrer dans les égalités $^1/_2 (x + a)$, $^1/_2 (x + b)$ etc. les valeurs génératives calculées, pour voir si les nombres ainsi obtenus s'accordent avec les moyennes trouvées par l'examen de la descendance. En procédant de cette façon, nous obtiendrons en même temps une autre vérification de l'exactitude des valeurs génératives trouvées.

Nous arrivons ainsi au tableau suivant (p. 24).

On voit que la différence des valeurs calculées et de celles observées ne s'élève dans aucun cas à plus d'environ $^1/_{25}$ vertèbre. Nous croyons avoir ainsi réalisé une concordance très suffisante, qu'on obtiendra difficilement plus satisfaisante dans les expériences biologiques de ce genre, où il est difficile, ou plutôt tout à fait impossible, de procurer à tous les échantillons de descendance les conditions extérieures rigoureusement identiques qui, en théorie, constituent la base de nos considérations.

Comparaison entre les valeurs observées et les valeurs calculées de la descendance.

Combinaison	Observé	Calculé	Différence	Erreurs probables dans les valeurs observées
xa	**58.519**	**58.555**	— 0.036	0.020
xb	**58.988**	**59.012**	— 0.024	0.023
xc	**57.870**	**57.850**	+ 0.020	0.020
xd	**58.229**	**58.188**	+ 0.041	0.018
ya	**59.400**	**59.364**	+ 0.036	0.017
yb	**59.846**	**59.822**	+ 0.024	0.023
yc	**58.640**	**58.660**	— 0.020	0.044
yd	**58.957**	**58.998**	— 0.041	0.019

Nous sommes maintenant en droit d'affirmer que l'examen plus approfondi de notre expérience a donné le même résultat que celui obtenu par le traitement préliminaire exposé dans le chapitre précédent (pp. 15—19), où nous nous sommes bornés à classer simplement les mâles, d'une part, et, de l'autre, les femelles, classification dont nous avons ensuite fait vérification. Dans l'un et l'autre cas, la justesse de nos réflexions s'est trouvée confirmée et, par là, il a été démontré que la Méthode du Croisement diallèle est bien applicable à ce genre de recherches.

Dans la détermination que nous venons d'effectuer des valeurs génératives des six individus en expérience, ce qui est particulièrement intéressant, c'est qu'elle a permis d'entreprendre une classification et une comparaison non seulement, comme auparavant, des mâles et des femelles séparément, mais aussi de plusieurs individus de sexe opposé.

Ce résultat présente un intérêt particulier lorsqu'il s'agit d'un caractère inhérent à l'un des deux sexes seulement. Chez le houblon, par ex., la substance résineuse active dans la fabrication de la bière, la lupuline, ne se rencontre guère que dans les inflorescences femelles; néanmoins, notre méthode nous permet d'apprécier deux ou plusieurs plantes mâles d'après leur teneur en lupuline malgré le fait qu'aucune d'elles ne contient jamais de lupuline comme les plantes femelles. Dans ces cas-là, la différence entre la valeur individuelle et la valeur générative se manifeste de façon particulièrement frappante.

IV. Combinaison des Méthodes des Lignes Pures et du Croisement diallèle.

Il est dans la nature de la chose que la Méthode des Lignes Pures n'est point applicable à la détermination de la valeur générative chez les animaux et plantes allogamiques. Par contre, la Méthode du Croisement diallèle n'est sujette à aucune limitation à cet égard. Partout où les circonstances le permettent d'ailleurs, cette dernière méthode peut s'appliquer aussi bien aux espèces allogamiques qu'aux autogamiques.

Chez les espèces autogamiques, on pourra ainsi déterminer la valeur générative de deux manières essentiellement différentes, à savoir par autofécondation et aussi par croisement. Une pareille combinaison des deux méthodes peut évidemment présenter beaucoup d'intérêt, soit au point de vue purement théorique, soit en nous fournissant le moyen d'exercer un double contrôle de l'exactitude de nos déterminations.

Supposons, pour fixer les idées, que d'une espèce végétale autogamique nous ayons trois exemplaires, *A*, *B* et *C*, appartenant chacune à sa race (ligne) et différant l'un de l'autre sous le rapport d'un caractère quantitatif quelconque, le poids des graines, par ex., ou la dimension des fleurs. Faisons reproduire ces trois végétaux soit par autofécondation, soit par croisement, et laissons leur descendance se développer dans des conditions extérieures identiques autant que possible. Désignons les valeurs génératives par *Ga*, *Gb*, *Gc*. Après autofécondation, nous aurons alors:

$$Ga = \text{Descendance } A$$
$$Gb = \text{Descendance } B$$
$$Gc = \text{Descendance } C,$$

«Descendance *A*» étant la moyenne, trouvée par un examen, de la descendance de la plante *A*, et ainsi de suite.

Croisons ensuite *A* avec *B*, *B* avec *C*, et *A* avec *C*. Ces croisements peuvent s'opérer de deux manières différentes: ou bien, par ex., en faisant servir *A* de mère et *B* de père, ou bien en employant, inversement, *B* comme mère et *A* comme père; en fait, un tel croisement en sens inverse (croisement réciproque) donne généralement le même résultat que le premier. Après avoir réalisé les croisements et laissé la progéniture se développer

dans les mêmes conditions que celle produite par l'autoféconda-tion, nous aurons:

$$^1/_2\ (Ga + Gb) = \text{Descendance } 1$$
$$^1/_2\ (Ga + Gc) = \text{Descendance } 2$$
$$^1/_2\ (Gb + Gc) = \text{Descendance } 3.$$

La «Descendance 1» est ici la moyenne de la progéniture née du croisement de *A* et de *B*, moyenne obtenue par l'examen de cette progéniture.

En soustrayant l'équation 1 de l'équation 2, on aura:

$$^1/_2\ (Gb - Gc) = \text{Descendance } 1 - \text{Descendance } 2,$$

et en faisant l'addition de cette équation et de l'équation 3:

$$Gb = \text{Descendance } 1 - \text{Descendance } 2 + \text{Descendance } 3.$$

D'une manière analogue, on peut déterminer *Ga* et *Gc*, et le résultat sera:

$$Ga = \text{Descendance } 1 + \text{Descendance } 2 - \text{Descendance } 3,$$
$$Gb = \text{Descendance } 1 - \text{Descendance } 1 + \text{Descendance } 3,$$
$$Gc = \text{Descendance } 2 - \text{Descendance } 1 + \text{Descendance } 3.$$

Nous avons ainsi déterminé les valeurs génératives des trois plantes aussi bien d'après la Méthode des Lignes Pures que par celle du Croisement diallèle, et il nous incombe maintenant de vérifier si les valeurs trouvées s'accordent. Il sera évidemment d'un grand intérêt de mettre ainsi à l'épreuve la Méthode du Croisement diallèle; vu cependant que je n'ai pas pu jusqu'ici pratiquer ces expériences vérificatrices, j'ai cru devoir pour le moment me borner à l'exposé schématique qui précède et qui, je pense, suffira à expliquer le procédé qu'il conviendra de suivre pour combiner les deux méthodes.

Cependant, par un heureux hasard, j'ai reçu, grâce à M. le Dr O. Winge, un mémoire publié, il y a quelques années, par le savant américain Thomas Harper Goodspeed[1]), et qui contient des éléments suffisants pour un examen préliminaire du problème qui nous occupe. Goodspeed à examiné trois variétés d'une espèce de Nicotiane: *Nicotiana acuminata* (Grah.) Hook., qui

[1]) Thomas Harper Goodspeed: Quantitative Studies of Inheritance in Nicotiana Hybrids. III. (University of California Publications in Botany, Vol. 5, N° 6, 1915).

diffèrent l'une de l'autre par la dimension des fleurs. Au cours de ses recherches, effectuées pendant les années 1910 à 1913, il a mesuré plus de 13000 fleurs; il est à regretter, cependant, que le nombre des plantes examinées soit moins élevé qu'on n'aurait pu le souhaiter. Les trois variétés sur lesquelles portent ses recherches et que je nommerai *A*, *B*, et *C* (l'auteur lui-même les désigne par I, II, III), furent propagées soit par autofécondation, soit par croisement, après quoi les dimensions des fleurs de la descendanee furent mesurées. Je reproduirai ici les résultats qu'ont donnés les mesures du diamètre de la corolle, lequel se trouva varier très notablement d'une variété à l'autre. Je m'en tiendrai à l'année 1911, la seule où des mesures de la première génération hybride (*F1*) ont été effectuées. En faisant usage des mêmes symboles que ceux employés dans le schéma donné plus haut, je vais reproduire d'abord les mesures faites par GOODSPEED sur la descendance produite par autofécondation. Tous les nombres cités ci-dessous représentent des valeurs moyennes d'un grand nombre de mesures du diamètre de la corolle, en millimètres. L'auteur américain n'a pas spécifié dans chaque cas le nombre des fleurs mesurees, mais seulement celui des plantes examinées. Malheureusement, ce nombre est parfois assez restreint; je le mettrai entre parenthèses après chaque moyenne.

Pour la descendance produite par autofécondation, GOODSPEED a trouvé en 1911 les valeurs suivantes:

$Ga = 27.20$ mm (5 plantes),
$Gb = 19.99$ · (7 plantes),
$Gc = 13.85$ · (5 plantes).

Pour la descendance produite par croisement (*F1*), il a trouvé dans la même année:

Pour $A \times B$: $^1/_2 (Ga + Gb) = 24.23$ (7 plantes),
Pour $A \times C$: $^1/_2 (Ga + Gc) = 20.92$ (17 plantes)[1]),
Pour $B \times C$: $^1/_2 (Gb + Gc) = 17.08$ (33 plantes)[1]).

[1]) Les valeurs numériques citées sont les moyennes des nombres de GOODSPEED qui, cependant, se présentent sous une forme tellement refondue et condensée, que je ne saurais garantir l'exactitude absolue de ces valeurs. Il aurait évidemment mieux valu que j'eusse eu à ma disposition les mesures originales au lieu de quelques valeurs moyennes dont on ne sait guère la sûreté avec laquelle elles ont été déterminées. (L'auteur dit à ce sujet: «The statistical constants for the tables which follow are not given, as their significance is doubtful with such small populations as were measured»).

A partir de ces trois équations, on peut, de la manière indiquée plus haut, trouver les valeurs de *Ga*, *Gb* et *Gc*. Elles sont inscrites dans la deuxième colonne du tableau ci-dessous, à titre de comparaison avec celles obtenues pour la descendance produite par autofécondation.

Valeurs génératives de *A*, *B*, et *C*, déterminées après autofécondation et croisement.

Symboles	Autofécondation	Croisement
Ga	27.20	28.06
Gb	19.99	20.40
Gc	13.85	13.75

En faisant la part des nombreuses sources d'erreur, dues en partie à ce que l'expérience mentionnée avait un autre but que celui qui m'a conduit à utiliser les résultats numériques auxquels elle avait abouti, j'estime que l'accord entre les déterminations de la valeur générative doit être reconnu comme étant à ce point satisfaisant, que la théorie de la Méthode du Croisement diallèle et, par suite, l'utilité de cette même méthode se trouvent confirmées de façon irrécusable[1]).

[1]) Il y a lieu de croire que l'accord précité eût été encore plus parfait, si le nombre de plantes examinées avait été plus grand. D'ailleurs, dans ces expériences, il est sans doute bien plus difficile que dans celles relatées plus haut sur les truites d'obtenir des moyennes parfaitement comparables pour les échantillons de descendance, attendu que, à la différence des jeunes poissons, les fleurs ne se développent pas synchroniquement, mais apparaissent successivement, de sorte que la diversité des conditions extérieures peut se faire sentir davantage.

Les mesures reproduites dans le texte ont toutes été effectuées en 1911. Pour l'année suivante, il n'a pas été publié de mesures complètes, mais bien pour 1913, année dans laquelle toutefois ce n'est plus la première, mais la troisième génération hybride (*F3*) qui a été examinée. En faisant le calcul pour cette génération, de la manière indiquée plus haut, nous arriverons, pour l'année 1913, aux résultats suivants:

Symboles	Autofécondation	Croisement
Ga	30.10	31.15
Gb	22.67	23.71
Gc	15.17	17.51

On voit par là que les nombres se rapportant à 1913 sont tous supérieurs à ceux obtenus pour 1911, ce qui montre que des caractères tels que la dimension des fleurs sont sujets à des variations considérables suivant les années.

Dès lors, je vois dans les remarquables expériences de GOODSPEED une nouvelle confirmation de la justesse des considérations énoncées dans le présent mémoire, et je suis porté à croire qu'une concordance encore plus parfaite pourra se réaliser quand on aura examiné sous ce rapport un encore plus grand nombre de plantes et qu'on sera parvenu à égaliser encore davantage les conditions extérieures. Alors la valeur moyenne de la descendance résultant de croisements se trouvera indubitablement coïncider avec celle des parents, et l'auteur que nous venons de citer ne pourra plus maintenir la thèse qu'il avance vers la fin de son mémoire, à savoir: «The mean flower-size may be approximately the mean of the parent flower-sizes, or it may not.»

Pour l'année 1911, nous avons trouvé que les valeurs déterminées par la méthode de croisement étaient: $Ga = 28.06$, $Gb = 20.40$, $Gc = 13.75$, ce qui donne $Ga + Gb + Gc = 62.21$. Les valeurs obtenues pour la descendance de plantes propagées par autofécondation, étaient: $Ga = 27.20$, $Gb = 19.99$, $Gc = 13.85$, donc $Ga + Gb + Gc = 61.04$. En diminuant dans la proportion de 61.04 : 62.21 les valeurs trouvées par la méthode de croisement, on aura:

	Symboles	Autofécondation	Croisement
	Ga	27.20	27.52
1911	Gb	19.99	20.02
	Gc	13.85	13.49
	Ga	30.10	29.24
1913	Gb	22.67	22 26
	Gc	15.17	16.44

Si enfin, pour donner à ces élements expérimentaux le plus d'ampleur que possible, nous prenons la moyenne des valeurs obtenues pour les deux années en question, nous aurons:

Symboles	Autofécondation	Croisement
Ga	28.65	28.38
Gb	21.33	21.14
Gc	14.51	14.96

En comprenant de cette façon dans nos calculs toutes les données à notre disposition, nous obtiendrons une concordance encore plus complète entre les nombres trouvés par les deux méthodes dont il s'agit.

Du reste, il n'est pas tout à fait incontestable qu'il soit légitime de coordonner les nombres relatifs aux deux groupes $F1$ et $F2$; mais il nous entraînerait trop loin d'entrer dans une discussion de ce point.

V. Remarques finales.

Dans ce qui précède, nous nous sommes occupés de l'hérédité des caractères quantitatifs, c'est-à-dire ceux qu'on peut compter, mesurer ou peser, et qui, par conséquent, sont susceptibles d'être exprimés par des valeurs numériques. La valeur d'un individu au point de vue d'une propriété quantitative quelconque peut, par suite, être dénotée simplement par un nombre, soit entier ou fractionnaire. De la même façon, la valeur de tout un groupe d'individus se laisse exprimer par un nombre, à savoir par la valeur moyenne de l'ensemble de ces individus.

La valeur trouvée par un examen direct (numération, mesurage, pesage) d'un individu, s'appelle la valeur personnelle ou individuelle de cet individu. Elle est à distinguer bien nettement de la valeur que l'individu présente à titre de générateur, sa valeur générative, laquelle nous ne pouvons arriver à connaître qu'en faisant l'examen de la descendance. Ce que nous entendons ici par valeur générative, ressort de l'équation

$$^1/_2\,(x + a) = \text{Descendance.}$$

où x et a signifient les valeurs des parents à titre de générateurs, et «Descendance» la valeur moyenne de leur postérité, — les conditions extérieures étant supposées constantes.

En réalité cependant, dans la nature, les conditions extérieures se maintiendront rarement constantes; à toute rigueur, elles ne le seront jamais, ni d'une génération à l'autre, ni même pour des individus ayant des parents communs et faisant partie d'une même portée. Cependant, la valeur personnelle d'un individu sera toujours influencée par la nature des conditions extérieures dans lesquelles il se développe. Il s'en suit que cette valeur personnelle ne coincide point nécessairement avec la valeur générative. Il peut même y avoir une différence assez notable, particulièrement frappante lorsqu'il s'agit d'un caractère qui ne se trouve que chez l'un des deux sexes.

De ce qui précède, il ressort que de la valeur personnelle d'un individu on ne saurait conclure à sa valeur générative.

Il est cependant souvent d'une grande importance, soit au point de vue théorique soit sous le rapport pratique, de connaître la valeur générative, par ex. lorsqu'il s'agit de la sélection d'individus destinés à la reproduction.

Quant à la manière dont on y arrive, nous l'avons indiquée aux chapitres précédents, où l'on a vu comment on peut évaluer un certain nombre d'individus selon leur valeur générative au point de vue de quelque caractère quantitatif.

En ce qui concerne les plantes autogamiques, on peut se servir à cet effet de la Méthode des Lignes Pures, tandis que celle du Croisement diallèle nous offre un moyen de détermination de la valeur générative chez les animaux et plantes allogamiques; cette dernière méthode peut du reste servir aussi lorsqu'il s'agit d'espèces autogamiques.

La Méthode des Lignes Pures a été brièvement esquissée au Chapitre II de ce mémoire (p. 3 à 4); pour des développements plus amples et plus détaillés, je dois renvoyer le lecteur aux écrits publiés depuis 1903 par W. JOHANNSEN[1]).

La Méthode du Croisement diallèle peut être succinctement caractérisée comme suit:

1° On réalise des croisements multiples (diallèles) en faisant accoupler chaque femelle avec chaque mâle. Soit A le nombre des femelles, et B celui des mâles; il résultera $A \times B$ différentes combinaisons de descendance.

2° On fait développer tous les échantillons de descendance dans des conditions extérieures identiques autant que possible.

3° Analyse (par numération, mesurage ou pesage) de la descendance, puis détermination de la valeur générative de chaque échantillon de descendance.

4° Calcul des valeurs génératives des parents. Puisque les échantillons de descendance, quoique présentant des moyennes différentes, contiennent les mêmes éléments diversement combinés, on est à même de classer ou déterminer par un calcul peu compliqué les valeurs génératives des parents.

Comme on le voit, le procédé à suivre pour le croisement diallèle n'est pas sans rappeler celui qu'on emploie si souvent dans les recherches chimiques: on fait «réagir» une substance composée sur d'autres, afin de découvrir quels sont les corps plus simples qui la composent. D'ailleurs, la Méthode n'est guère, en principe, différente de l'analyse de croisement usitée dans la génétique. La seule différence consiste, au fond, en ce qu'en

[1]) Parmi ceux publiés à l'étranger, il faut citer: «Ueber Erblichkeit in Populationen und in Reinen Linien», Iéna (Gustav Fischer) 1903; «Elemente der exakten Erblichkeitslehre», 2me édition, Iéna (Gustav Fischer) 1913.

faisant usage de cette méthode-là on opère uniquement avec les valeurs moyennes de la descendance. En conséquence, la Méthode du Croisement diallèle ne peut pas servir dans l'étude des caractères indéterminables par numération, mesurage ou pesage, tandis que ceux qu'il est possible de déterminer par l'une ou l'autre de ces opérations[1]) se prêtent bien à l'application de cette méthode.

Pour ce qui regarde l'application de la Méthode du Croisement diallèle, elle n'est — en théorie du moins — sujette à aucune limitation: elle peut s'appliquer à tous les animaux et à tous les végétaux à reproduction sexuelle. Les espèces qui s'y prêtent le mieux, sont celles qui se distinguent par une progéniture nombreuse et un développement rapide; pourtant, nos recherches sur les truites ont montré que, pour parvenir à une classification correcte des parents, on n'aura à examiner qu'un nombre relativement restreint d'individus de chaque combinaison de descendance. J'ai donc lieu d'espérer que cette méthode pourra rendre des services dans l'élevage des animaux domestiques et des plantes cultivées, en facilitant la sélection des individus reproducteurs, et que par suite elle contribuera à l'amélioration rationnelle des populations animales et végétales. Cependant, je ne veux rien prophétiser, et je me bornerai ici à exprimer le souhait que les hommes pratiques aussi bien que les hommes de science prennent intérêt à faire l'épreuve de la méthode. Il me paraît incontestable que les résultats obtenus ont prouvé la justesse du principe qui y préside, et je suis fondé à croire que ce principe se montrera valable pour la plupart des

[1]) Il en est ainsi, j'estime, de tous les caractères — «polymères» — qui dépendent de plusieurs facteurs scindant de façon indépendante et dont les effets s'accumulent (facteurs à «action égale»). A cette catégorie appartiennent probablement la plupart des caractères quantitatifs présentant des passages graduels entre les valeurs minima et maxima.

Par contre, la Méthode ne sera pas applicable aux cas où il y a dominance (citons, à titre d'exemple, le cas étudié par BATESON et GREGORY sur le genre *Primula*, où le type à style court prédomine sur celui à style long). En effet, l'application de cette méthode — au croisement de deux individus homozygotes, par ex. — est subordonnée à cette condition, que la valeur moyenne des parents dans des conditions extérieures égales coïncide avec la valeur moyenne de la totalité des descendants faisant partie soit de la première, soit de la seconde génération hybride (*F 1* et *F 2*). (Pour les notions de «dominance» et de «facteurs à action égale«, voir par ex. le livre de W. JOHANNSEN paru en 1913 et cité p. 31, en note).

propriétés quantitatives des animaux et des plantes à reproduction sexuelle; mais il reste à prouver que la Méthode est capable de fournir des résultats de valeur générale. Il s'agit donc de l'éprouver dans la pratique, en faisant l'application à autant d'espèces et de caractères que possible[1]). L'affaire est bien simple en elle-même, et j'estime que la suite d'idées sur laquelle la Méthode est basée n'est point étrangère aux hommes pratiques; en effet, bon nombre de ceux-ci s'appliquent probablement, de façon plus ou moins consciente, à réaliser ce que dans les pages précédentes j'ai appelé croisement diallèle. Ce qu'il y a de nouveau dans l'affaire telle que je viens de l'exposer, ce serait donc le mode d'application rationnel et raisonné que j'en ai pu formuler nettement après avoir eu la bonne fortune de trouver dans le nombre de vertèbres de la truite un élément propre à mettre en lumière toute cette affaire. Je crois être en droit d'appeler cela une bonne fortune, attendu que, à ma connaissance, on trouvera difficilement, soit dans le règne animal soit parmi les végétaux, beaucoup d'espèces ou de caractères qui se prêtent mieux que cet élément-là au traitement précis et exact qui seul permettra de séparer la paille d'avec le bon grain et de mettre en évidence, affranchies, les lois auxquelles obéissent les phénomenes observés[2]). Une fois qu'on aura l'intelligence de ce qui est l'essentiel, il ne sera plus difficile de retrouver la loi, même dans les cas -- et ils sont extrêmement fréquents — où elle apparaît plus ou moins voilée par suite de perturbations diverses.

[1]) A ce propos, je ferai remarquer que notre Laboratoire Carlsberg a déjà fait des démarches utiles à cet effet, pour ce qui concerne plusieurs animaux domestiques et plantes cultivées de ce pays.

[2]) Citons seulement, à ce sujet, la grande fécondité de la truite, les chances qu'elle fournit de réaliser sur une grande échelle des croisements diallèles et de faire développer synchroniquement les descendants; enfin, le nombre peu variable des vertèbres, lesquels on peut compter avec une certitude absolue.

Laboratoire Carlsberg, à Copenhague, 3 mars 1919.

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RECHERCHES SUR LES ORGANISMES DE FERMENTATION

PAR

ALB. KLÖCKER.

IV. CONTRIBUTION A LA CONNAISSANCE DE LA FACULTÉ ASSIMILATRICE DE DOUZE ESPÈCES DE LEVURE VIS-A-VIS DE QUATRE SUCRES.

1. Introduction

En formant le plan des recherches qui font l'objet de ce mémoire, j'avais d'abord l'intention de les faire porter sur un plus grand nombre de levures et de sucres que celui auquel je me suis arrêté en définitive, savoir 12 de celles-là et 4 de ceux-ci. Cependant, je me suis bien vite aperçu que le travail ne pourrait être achevé que dans un avenir assez lointain, s'il devait s'exécuter suivant ce plan-là. Je m'étais, en effet, proposé de déterminer par numération la multiplication et par là la faculté d'assimilation et, d'autre part, d'utiliser plusieurs sources d'azote différentes. Comme cependant les numérations que comporte une quelconque de ces expériences doivent nécessairement être effectuées par une seule et même personne, sans quoi l'on ne peut obtenir des résultats parfaitement sûrs et qui puissent être comparés les uns avec les autres, il a fallu me limiter au nombre sus-indiqué de levures et de sucres. Quant à la méthode que j'ai suivie, on la trouvera décrite plus bas; qu'il suffise de mentionner ici que, malgré les limitations indiquées, le nombre de numérations auxquelles mes recherches ont donné lieu, s'élève à 20 000 environ.

Cependant, je me suis heurté à certaines difficultés de nature diverse, qui ont eu pour conséquence que la contribution que j'ai pu fournir à la solution du problème en question est moins complète que je ne l'aurais souhaité. En effet, je m'étais placé à ce point de vue indiscutable en soi, que la multiplication des cellules de levure dans un liquide nutritif quelconque correspond à l'assimilation des substances chimiques dont se compose ce liquide. Mais il faut remarquer qu'il entre en jeu un si grand

nombre de facteurs très divers et en partie très importants que, toutes les fois qu'il s'agit d'une assimilation faible, les choses se compliquent singulièrement. Aussi rencontre-t-on, parmi les données bibliographiques, des affirmations grossièrement discordantes, soit que les différents chercheurs aient employé des méthodes différentes, soit qu'ils aient omis de faire entrer en ligne de compte certains facteurs qui pourtant ne sont pas sans avoir leur importance, tels que, pour en citer un exemple, le degré de pureté des sucres concernés. Un autre facteur dont on a parfois également négligé de tenir compte, est la teneur de l'air en matières azotées.

Avant de passer à l'exposé de mes recherches personnelles, je vais mentionner brièvement les publications les plus marquantes qui, à ma connaissance, ont paru jusqu'à présent dans ce domaine.

En 1902, Bokorny (1) avait donné une communication sur l'influence exercée par divers facteurs extérieurs sur la faculté assimilatrice de la levure. Il trouve la «grandeur de l'assimilation» en déterminant le poids de la levure sèche. Cependant, il est évident que de cette façon on n'arrive pas à un résultat exact. En effet, une multiplication faible ne saurait être évaluée par le pesage. D'ailleurs, une même quantité de levure en poids peut contenir un nombre extrêmement différent de cellules, suivant que celles-ci sont plus ou moins grandes ou petites. Il faut remarquer en outre, que cet auteur employait de la «levure pressée» («Presshefe»), désignation très vague qu'il n'a pas précisée davantage. En somme, ses recherches n'ont guère d'importance, ce qui me dispense d'entrer dans leur détail.

En 1905, Lindner (1) publia un mémoire traitant de la faculté qu'ont les levures et d'autres champignons d'assimiler leurs produits d'autodigestion. Le procédé qu'il employait consistait à appliquer, à l'aide d'un pinceau, la levure délayée sur la surface d'agar nutritif chargé des divers produits de décomposition. Dans ses expériences plus récentes, toutefois, il a abandonné ce procédé qui, du reste, doit sans doute être considéré comme peu sûr, attendu qu'il est très difficile, sinon impossible, de vérifier s'il y a une augmentation de croissance sur le milieu de culture lorsque celui-ci renferme les produits d'autodigestion.

Pringsheim (1) recourt à la numération dans ses investigations sur la nutrition azotée de la levure. Le nombre de cellules développées au cours de la fermentation est déterminé comme

s'élevant à tant de millions par cm^3, puis les nombres obtenus sont comparés entre eux. Les recherches ainsi conduites n'ont cependant aucun intérêt, lorsqu'il s'agit d'examiner si une faible assimilation a eu lieu ou non.

Le premier travail d'importance qui a pour objet l'assimilation de différents hydrates de carbone par diverses espèces de levure, est le mémoire publié en 1910 par Lindner et Saito (1), qui donnent la description suivante de leur mode opératoire:

«Nous employions une dissolution, dans l'eau potable, de sulfate de magnésium, 0.025 pour cent — phosphate de potassium primaire, 0.5 p. c. — asparagine, 0.5 p. c. Cette liqueur nutritive fut additionnée des diverses sources de carbone, en quantité égale à 5 p. c. en poids .... Ces produits chimiques avaient tous été fournis par la maison Kahlbaum, à Berlin. 5 cm^3 de chaque liqueur nutritive furent mis dans un flacon, puis stérilisés .... Nous ensemencions avec des cultures fraîches provenant de la collection de notre Institut. La quantité ensemencée était aussi petite que possible, afin de nous permettre d'observer facilement la croissance éventuelle et, d'autre part, d'empêcher la croissance, si faible fût-elle, à laquelle un ensemencement plus fort aurait pu donner lieu (en distribuant l'azote sur un plus grand nombre de cellules). Les essais étaient faits à la température de 25° C., et les résultats furent notés au bout de 10 jours .... Toutes les fois qu'un développement pouvait être observé, il fallait en désigner aussi l'intensité, ce qui se faisait par les chiffres 1, 2 et 3.

Le chiffre 3 signifie qu'il s'était produit un développement si abondant, qu'en secouant le liquide on le voyait se troubler fortement. 2 signifie l'apparition d'un trouble moyen, 1 celle d'un trouble léger. Nous avons mis un ? dans tous les cas où il paraissait douteux que les petits flocons de levure observés provinssent d'un développement, ou bien au contraire qu'ils eussent été apportés par la levure ensemencée dans la liqueur. Le signe ÷ dénote l'absence de trouble et de développement ...»

Or, cette manière de déterminer la grandeur de l'assimilation ne donne pas non plus des résultats satisfaisants. En effet, ceux-ci dépendent trop d'une appréciation personnelle; les auteurs eux-mêmes le reconnaissent dans une certaine mesure. Aussi, même en m'attachant à suivre exactement le même procédé, je suis arrivé, dans bien des cas, à des résultats qui ne concordent pas avec ceux obtenus par ces deux savants.

Rose (1) a effectué des essais d'assimilation avec l'Endomyces Magnusii sur un milieu de culture composé d'eau potable, de gélatine (12 p. c.) et de liquide nutritif Hayduck, sans addition d'aucune source particulière d'azote. Après avoir ajouté à la gélatine 10 p.c. de sucre, il versait le mélange dans des boîtes Petri. La levure délayée fut, à l'aide d'un pinceau, appliquée à la surface de manière à y former des stries.

Dans un mémoire plus récent, Lindner (2) indique qu'il soumet le liquide nutritif à la centrifugation, pour peser la levure ensuite. Cette manière d'agir manque pourtant de précision, puisqu'il pèse la levure à l'état humide.

Tor Carlson (1), à son tour, évalue la quantité de levure après centrifugation, en faisant usage de verres dont l'extrémité inférieure est étirée en forme d'un tube capillaire gradué permettant de lire la quantité cherchée. Le résultat ainsi obtenu ne peut cependant être tout à fait sûr parce que, toutes les fois qu'il s'agit d'une multiplication faible, il est impossible de constater la différence. Il convient de remarquer en outre que, de même que dans les essais de Bokorny, le résultat est influencé par la dimension des cellules.

Waterman (1) fait subir la polarisation à la solution nutritive, à l'effet de déterminer la quantité de sucre disparu et, partant, l'étendue de l'assimilation. La solution était un mélange d'eau potable (dont, selon lui, la teneur en azote n'est pour rien dans la croissance de la levure) avec du dextrose (2 p. c.), du phosphate de potassium primaire (0,2 p.c.), du sulfate de magnésium (0,1 p.c.) et des composés azotés (1 p.c.). Il n'opère que sur de la «levure pressée» («Presshefe»).

Kita (1) a essayé de déterminer l'assimilation par numération, de même que l'a fait l'auteur du présent mémoire; mais ses essais qui, du reste, ne portent que sur une seule espèce, manquent de netteté.

Dans un travail récent, Bokorny (2) a également employé la numération, ainsi que l'évaluation du poids de la levure sèche. Ses numérations ne semblent cependant pas être tout à fait sûres, puisque le nombre de cellules qu'il trouve au bout de deux jours est moindre que celui des cellules ensemencées, et cela malgré un poids sec plus grand.

Nous allons maintenant considérer d'un peu plus près les différents facteurs dont l'influence est prépondérante dans toutes

recherches de ce genre. Parmi ces facteurs, il convient de mentionner tout d'abord la quantité de levure ensemencée.

En 1901, Wildiers (1) avait publié un mémoire dans lequel il parle d'une matière jusque-là inconnue qu'il appelle «Bios». Dans 125 cm^3 d'une dissolution de sels minéraux et de sucre, dans laquelle l'azote ne se trouvait qu'à l'état de chlorure d'ammonium, il sema une très faible quantité de cellules de levure, et n'obtint ni fermentation ni propagation de la levure; au contraire, il y arriva en ajoutant de l'eau de levure. Il constata, en outre, que cette dernière pouvait être remplacée par l'extrait de viande, la peptone ou le moût de bière. Il estime, en conséquence, que la levure ne peut se contenter d'azote inorganique, et qu'il faut une certaine proportion d'une substance jusqu'alors inconnue qu'il nomme *Bios*. La levure elle-même contient de cette substance, qu'elle consomme au cours de la fermentation; mais elle n'en produit pas de nouvelles quantités. C'est de la quantité développée d'acide carbonique que Wildiers tira ces conclusions; mais il ne comptait pas les cellules semées et récoltées. Il ne rencontra pas de Bios dans l'urée, l'asparagine, la tyrosine etc. Le Bios, dit-il, était insoluble dans l'alcool absolu et l'éther, alors qu'il se dissolvait dans l'alcool à 80 pour cent.

Krieger (1) estime que la levure doit pouvoir produire du Bios parce qu'une décoction de levure, l'eau de levure, en renferme une quantité abondante; selon Krieger, il doit se former, au cours de la fermentation, du Bios en proportion correspondent à la propagation de la levure. Quant à la question de savoir si le Bios existe préformé dans la levure, ou s'il n'est formé qu'à la suite de la cuisson de la levure (opération nécessaire pour la préparation de l'eau de levure), il ajoute qu'elle n'est pas encore résolue[1]).

Fernbach (1) est d'avis que la raison par laquelle la levure ne se développe pas lorsqu'on n'en ensemence qu'en faible proportion, doit être cherchée dans ce fait, que le liquide nutritif contiendrait de faibles quantités d'une substance douée de la propriété de paralyser la levure, substance qui, si elle n'est pas un vrai antiseptique, en exercerait du moins l'action.

Henry (1), effectuant un certain nombre d'essais sur diverses espèces de levure, trouva que les résultats démentaient l'assertion

[1]) Il se pourrait pourtant que le Bios soit communiqué à la levure par un liquide nutritif qui en contiendrait.

de Wildiers concernant la présence de Bios. D'autres expériences démontrèrent que, à supposer que cette substance existe réellement, il doit pouvoir s'en former dans les cellules de levure elles-mêmes.

Windisch (1) ne croit pas à l'existence du Bios. Si la levure ne se développe pas lorsqu'elle est ensemencée en quantité très faible, cela tient selon lui à la présence dans le milieu nutritif de certaines substances exerçant une action antiseptique, des toxiques. Bien qu'elles ne s'y trouvent qu'en quantité minime, elles sont susceptibles d'entraver la croissance de la levure lorsqu'on n'a ensemencé qu'un petit nombre de cellules. Windisch dit même avoir observé il y a plusieurs années, que la faible quantité de bleu d'outremer parfois contenue dans le sucre agit comme un poison sur la levure. Windisch renvoie, en outre, aux recherches de Nägeli, qui montrent que même les doses fort minimes de sels métalliques qui apparaissent quelquefois dans l'eau distillée produisent des effets antiseptiques. Si, malgré cela, une trace de levure ensemencée dans du moût de bière peut se propager, cela ne tient point, comme Wildiers croit, à ce que le moût contiendrait du Bios. Le moût contient, à la vérité, de petites doses de cuivre qui, suivant Nägeli, auront pour effet de rendre le liquide oligodynamique; mais le moût renferme aussi des matières albuminoïdes, de la gomme, de la dextrine etc., donc des substances colloïdales qui neutralisent l'action oligodynamique du cuivre.

L'importance de la dose de levure ensemencée a aussi été signalée par Kossowicz (1). Il ensemença 200 cellules dans une dissolution de sels minéraux additionnée de sucre; au bout de cinquante jours, elles s'étaient multipliées au point d'atteindre le nombre de 140 millions. Lorsque, au contraire, il ne semait qu'une seule cellule, il n'obtenait point de développement. Dans un seul essai sur vingt-deux faits avec une cellule unique, il vit se produire un développement; mais celui-ci était probablement dû à un peu de matière nutritive qui aurait été entraînée dans la solution.

Amand (1) constata que dans des liquides nutritifs où il avait semé de la levure, la teneur en Bios diminuait rapidement et ne pouvait plus être retrouvée par la lixiviation des cellules.

Kita (1) estime que la maltose non purifiée renferme une substance identique à l'oryzanine isolée par Suzuki du son de

riz, substance qui se comporte comme le Bios, en ce sens qu'une faible teneur dans un liquide nutritif artificiel augmente l'assimilation de la maltose.

Bokorny (2) montre qu'il doit y avoir un certain rapport entre la proportion de levure et celle de sucre. Quand on ensemence trop de levure (soit 2 g de levure pour chaque 2 g de sucre), on obtient peu ou pas de développement, résultat qu'il explique en admettant que dans ces conditions tout le sucre est fermenté si rapidement qu'il n'en reste guère pour la fermentation. Le rapport le plus favorable se trouva être 0g,02 de levure pour 2 g de sucre.

De ce qui précède il ressort qu'il est indispensable de tenir compte de la quantité de la levure ensemencée; les résultats discordants auxquels les différents auteurs sont arrivés s'expliquent même dans bien des cas en admettant qu'ils ont négligé de prendre en considération ce facteur.

Comme un autre agent qui a également une certaine influence — pas très grande, il est vrai —, il convient de citer la lumière. On sait depuis bien longtemps que la lumière tend plutôt à entraver la propagation. Buchta (1) trouva que la «levure ordinaire de bière» se multipliait, toutes choses égales d'ailleurs, environ deux fois aussi vite à l'obscurité que lorsque les cultures étaient exposées à l'action de la lumière. Plusieurs années auparavant, Zikes (1) était arrivé au même résultat, ainsi qu'il en fait mention dans un compte rendu du travail de Buchta.

Une influence vraiment considérable est exercée, au point de vue du développement des levures, par les composés azotés faisant partie du milieu nutritif. Il en est de même, jusqu'à un certain point, de ceux existant dans l'air. Il est vrai qu'un certain nombre d'affirmations d'après lesquelles les Saccharomycètes auraient la faculté de s'assimiler l'azote libre de l'air se sont montrées être la suite d'une erreur; la propagation était en réalité due à des impuretés contenues dans les sucres. Kossowicz (2), par exemple, dit: «Ces recherches m'ont fourni la preuve rigoureuse de ce fait, que les Saccharomycètes . . . peuvent, eux aussi, s'assimiler l'azote atmosphérique libre». Cependant, il n'a pas analysé le sucre employé sous le rapport d'une teneur possible en azote et, par suite, il se trouve plus tard (3) obligé d'annuler sa preuve «rigoureuse», parce qu'il arrive à ce résultat que le sucre sur lequel il avait opéré n'était pas exempt d'azote et que la

levure peut bien utiliser les doses minimes de composés azotés existant dans l'air, mais non pas l'azote libre.

Lipman (1), à son tour, trouve que les composés azotés de l'air sont susceptibles d'être utilisés par les Saccharomycètes. Il est vrai qu'il emploie l'expression «atmospheric nitrogen», mais le contexte montre que par là il entend les composés azotés de l'air.

Will (1) observa que dix expèces de Torula possédaient la faculté de s'assimiler les composés azotés de l'air. Lui aussi dit: «Stickstoff», mais entend composés azotés.

Au reste, les nitrates ne sont censés avoir aucune valeur pour les Saccharomycètes (Waterman (1)); bien plus, on a prétendu qu'ils sont susceptibles de se réduire à des nitrites toxiques et de devenir ainsi nuisibles.

Duclaux (1) a démontré que dans de certaines conditions la levure préfère les sels ammoniacaux comme source d'azote. Les composés ammoniacaux existant dans l'air pourront dès lors acquérir de l'importance pour la multiplication.

Artari (1) trouva que la source d'azote la plus favorable pour le Saccharomyces Zopfii étaient des sels ammoniacaux.

En somme, les substances azotées faisant partie du milieu nourricier jouent un rôle très important. Quant à ce qui concerne celui qui est dévolu à chacun de ces composés en particulier, les opinions sont grossièrement discordantes. Il est certain que leur influence sur les diverses espèces est différente. C'est ce qui ressort des données bibliographiques que nous allons citer.

Schjerning (1) établit que la multiplication végétative peut avoir lieu lors même que le liquide nutritif ne contient aucune des substances azotées que le microorganisme dont il s'agit peut faire servir à sa nutrition; c'est dire que la multiplication peut se réaliser même en l'absence de l'azote. A ma connaissance, Schjerning est le seul auteur qui ait exprimé cette manière de voir. Il dit en outre: L'asparagine ne peut pas servir d'aliment azoté aux Saccharomyces Carlsbergensis ou S. apiculatus, et ces espèces ne sont pas à même de la transformer de manière à la rendre utilisable. Elles se sont bien multipliées, mais seulement pendant les premières vingt-quatre heures. Les cellules nouvelles ne contenaient point d'azote; somme toute, l'entière quantité de levure n'en renfermait pas plus que les cellules ensemencées[1]).

[1]) Comme on le verra plus loin, j'ai constaté, au contraire, que le S. Carlsbergensis peut se multiplier abondamment en se servant de l'asparagine comme source d'azote.

Pour le Schizosaccharomyces octosporus, Beyerinck (2) remarqua qu'il ne peut, lui non plus, se servir de l'asparagine (ni des sels d'ammonium). Je suis arrivé au même résultat, d'accord avec Lindner (2). Ce dernier dit que l'asparagine n'est nullement propre à servir de source d'azote (3), nonobstant qu'il ait affirmé auparavant avoir obtenu un développement dans une solution d'asparagine sans sucre (4), affirmation à laquelle il ajoutait que l'asparagine peut se présenter comme source d'azote et de carbone à la fois. Selon Kluyver (1), l'asparagine n'est pas une source d'azote utilisable, et Davis (1) est du même avis.

Plusieurs chercheurs ont trouvé que l'asparagine est une excellente source d'azote pour la «levure» (aucun d'eux n'indique l'espèce sur laquelle il a opéré); ce sont Hayduck (1), Laurent (1), Heinzelmann (1), Kusserow (1) et Wijsmann (1); Waterman (1) est arrivé au même résultat.

Beyerinck (1) dit: «Il suffit de relever que les levures de vin et de bière sont astreintes à recourir aux amides, comme par exemple à l'asparagine (mais non à l'urée), et surtout aux peptones».

Pringsheim (1) observa que pour la multiplication il y a dans certains cas un maximum de concentration d'azote; pour l'asparagine, par ex., dans des essais avec la levure dite Logos, ce maximum était situé à 1g,718 par litre: il trouva en ce cas, par cm^3, 52,4 millions de cellules de cette levure, alors que deux fois autant d'asparagine n'en donna que 42,8 millions, et qu'une quantité moitié moindre en produisit 40,2 millions. Dans le cas de la peptone, le nombre de cellules s'accrut avec la quantité de peptone. Cette substance se montra, envers la levure Logos, être une source d'azote beaucoup meilleure que l'asparagine.

Lindner et Wüst (1) trouvèrent que les levures peuvent assez bien user de l'urée comme source d'azote.

En ce qui concerne l'assimilation des divers sucres par les levures, on trouve dans la littérature les indications suivantes.

Beyerinck (1) affirme que la faculté assimilatrice n'est pas un phénomène vague ou douteux, mais au contraire — pourvu que les expériences soient conduites de façon convenable — un phénomène aussi net et aussi sûr que les réactions chimiques les mieux connues. S'il en était réellement ainsi, on ne rencontrerait pas dans la littérature tant d'indications contradictoires. D'ailleurs, on peut se demander ce qu'il faut entendre par «expériences conduites de façon convenable». Celles de Beyerinck ont porté sur

les «espèces de levures» indiquées ci-dessous, lesquelles cependant ne sont point des espèces bien définies qui se trouveraient décrites dans la littérature. Ainsi par ex., personne ne sait ce que sont le Saccharomyces Pastorianus Reess et le S. Pastorianus Pasteur.

Dans l'énumération qui suit, M signifie maltose, D dextrose, S saccharose, et L lactose; + dénote qu'il y a assimilation, *i*, que la levure peut intervertir la saccharose.

Voici les résultats de Beyerinck:

S. ellipsoideus (levure de vin ou levure pressée): M +, D +, S +, *i*. C'est une invention de Beyerinck que de ranger la levure pressée dans le groupe de S. ellipsoideus.

S. cerevisiæ (levure de bière): M +, D +, S +, *i*.

S. Pastorianus Reess: M +, D +, S +.

S. fragrans (S. Pastorianus Pasteur) D +, S +.

S. Kefyr (levure des grains de képhyr): D +, S +, L +, *i*.

S. Mycoderma: D +.

S. acetæthylicus (levure d'éther acétique): M +, D +, S +, *i*.

Dans un travail ultérieur (2), il dit que le Schizosaccharomyces octosporus assimile la dextrose et la maltose, mais non la saccharose.

Artari (1) indique que le Saccharomyces Zopfii peut assimiler la dextrose et la saccharose, mais non la maltose. Je crois devoir citer ce résultat parce que c'est ordinairement par exception que la maltose n'est pas assimilée. Je n'ai pas fait d'expériences personnelles sur cette espèce.

Dans le grand mémoire de Lindner et Saito (1), on trouve les faits suivants: Les levures de bière à fermentation basse ne donnent avec la dextrose qu'un développement faible ou moyen. En général, elles ne s'assimilent pas la saccharose. Dans la maltose elles se développent d'ordinaire abondamment; ce sucre est la matière plastique la mieux appropriée à l'édification du corps cellulaire. Avec la lactose on n'observe qu'un développement plutôt douteux. Quant aux levures de bière à fermentation haute, on est étonné d'observer très fréquemment qu'elles ne se développent point dans la dextrose et la saccharose. La maltose donne généralement une croissance plus faible que celle de la levure de bière à fermentation basse: les levures hautes ne s'y développent parfois même que très faiblement ou pas du tout. La lactose n'est presque jamais assimilée. La levure de vin atteint dans la dextrose une croissance moyenne; dans la saccha-

rose elle ne se développe que faiblement (les auteurs citent même un cas où le développement était nul). L'assimilation de la maltose est le plus souvent très forte, bien que certaines espèces ne l'assimilent que peu ou médiocrement. La lactose ne provoque qu'une croissance douteuse. Soumise à l'action de vingt-quatre espèces différentes de levures sauvages, la dextrose fut assimilée moyennement par dix d'entre elles, tandis que pour onze de ces levures l'assimilation était faible, pour deux douteuse et pour une, même nulle. Celle de la maltose était en règle générale très forte; dans cinq cas seulement on observa une assimilation moyenne et dans trois une assimilation faible. Dans la saccharose, la croissance était très forte pour six espèces, moyenne pour cinq, faible pour huit, douteuse pour trois. Dans la lactose, elle était nulle dans deux cas, faible dans trois, douteuse dans tous les autres.

Les deux auteurs résument comme suit les principaux résultats de leurs recherches:

Parmi les différents sucres expérimentés, la maltose est celui qui est le mieux approprié à l'assimilation; dans de très rares cas seulement, elle n'est assimilée que peu ou pas du tout.

La lactose représente l'extrême opposé: ce n'est que par exception qu'elle est assimilée.

La saccharose, si facile à faire fermenter, n'entre que pour peu de chose dans l'alimentation des levures; à part les levures sauvages, elle est sous ce rapport inférieure même à la dextrose.

La dextrose n'est assimilée en général que médiocrement, quelquefois pas du tout.

Le Schizosaccharomyces octosporus était la seule levure qui, l'asparagine lui servant de source d'azote, ne puisse s'assimiler aucun des sucres considérés.

Le Saccharomycodes Ludwigii, dans les mêmes conditions, ne peut s'assimiler qu'un peu de maltose.

Les auteurs ajoutent qu'avec d'autres sources d'azote on arrivera peut-être à des résultats différents, particulièrement pour ce qui concerne le S. octosporus. L'exposé de mes recherches va montrer qu'ils ont raison.

Rappelons que dans les expériences que je viens de résumer la source d'azote employée était formée uniquement d'asparagine.

Kita (2), ayant expérimenté sur quatre formes de Torula, dit que la maltose, contrairement à la dextrose, est favorable à

la croissance. Lui aussi avait ajouté de l'asparagine à la solution nourricière.

Kluyver (1) trouva que la maltose fournie par Kahlbaum et employée par Lindner renfermait 0,22 pour cent de matière albuminoïde, alors qu'après purification elle se trouva n'en contenir que 0,04 p. c.; la dextrose en contenait 0,02 p. c. En se servant de dextrose et comme source d'azote d'eau de levure, il obtenait un bon développement; dans l'eau de levure seule, au contraire, l'évolution était très réduite. L'emploi de la maltose non purifiée lui donna le même résultat que celui obtenu par Lindner. Ce dernier (2), au sujet des diverses publications relatives à l'assimilation de l'azote libre de l'air, fait remarquer que des expériences faites par Kluyver avec la maltose de Kahlbaum il résulte qu'il faut pour le moment en demeurer là pour ce qui regarde les levures. Et il ajoute: «de même pour l'asparagine, attendu que celle-ci mêlée à du sucre pur ne donne point de croissance et, partant, n'est pas assimilée». Comme nous l'avons vu plus haut, il a lui-même obtenu du développement dans une solution d'asparagine exempte de sucre (4).

Davis (1) confirme le résultat obtenu par Kluyver quant à l'azote dans la maltose non purifiée. La maltose soi-disant pure de Kahlbaum renferme 5 à 15 p. c. de dextrine et de faibles quantités de dextrose. Aucune des différentes sortes qui sont dans le commerce, et qui toutes sont prétendues pures, ne l'est en réalité. Pour éliminer la dextrose de la maltose, on peut se servir du Saccharomyces Marxianus, parce que cette levure ne s'assimile généralement pas la maltose. Il convient de remarquer toutefois, que cela dépend de la source d'azote utilisée; en effet, comme on le verra plus bas, j'ai constaté que dans certains cas la levure en question peut bien s'assimiler un peu de maltose.

Will (2), qui soumit à l'analyse la maltose la plus pure de Merck, n'y rencontra ni azote ni dextrose. Il la fit recristalliser aussi bien dans l'alcool éthylique que dans l'alcool méthylique. Lorsque dans la maltose du commerce, même dans la plus pure, une quelconque des quatre espèces de Pseudosaccharomyces expérimentées par Will arrive à se multiplier, c'est grâce à une substance contenue dans la préparation, substance exempte d'azote, réduisant la liqueur de Fehling et plus facilement soluble dans l'alcool éthylique que dans l'alcool méthylique. Il se peut que cette substance ne prenne naissance que sous l'influence de la

température assez élevée employée pour la stérilisation de la solution de maltose.

Citons enfin, que Lindner (5) a pratiqué un certain nombre d'expériences en vue de rechercher si les levures sont susceptibles de s'assimiler de l'alcool. A cet effet, il sema la levure dans une dissolution de sels inorganiques, en employant l'alcool comme source unique de carbone. Pour quelques-unes des levures mises en expérience, il put observer dans la dite solution un développement plus ou moins abondant. L'alcool est-il assimilé également en présence de sucre? L'auteur n'en dit rien et, à ma connaissance, il n'a jamais été publié d'essais sur ce point. Il semble pourtant absolument invraisemblable qu'au moins une partie du sucre ne subisse pas l'assimilation en même temps que l'alcool. Dans les cas où la croissance se produit dans une solution de sucre qui échappe à l'action fermentative de la levure considérée, cette croissance ne peut donc pas être due à une assimilation d'alcool. En ce qui concerne les autres cas relatés par Lindner, il est à remarquer que certains d'entre eux se rapportent à des espèces ayant formé des cellules de voile qui, on le sait, sont susceptibles d'oxyder l'alcool, espèces dont les végétations étaient plus âgées que celles sur lesquelles ont porté mes propres essais; ailleurs, la croissance observée était souvent si insignifiante que, l'augmentation de volume ayant été évaluée à vue d'œil, elle doit être regardée comme plutôt douteuse. Dans la suite j'ai cru pouvoir faire abstraction des résultats mentionnés en dernier lieu, et cela d'autant plus que Lindner lui-même les a passés sous silence en se référant, dans des publications ultérieures, à ces expériences-là.

2. Mes recherches

Pour les expériences dont on va lire l'exposé, je faisais usage de flacons Freudenreich, excepté lorsque l'air destiné à être conduit dans les flacons devait être débarrassé des matières azotées; en ce cas, j'avais recours à des ballons Pasteur. J'y semais toujours des cellules jeunes et vigoureuses de la levure concernée, cellules qui, à moins d'indication contraire, avaient été engendrées dans du moût de bière non houblonné. Après décantation du moût, la levure déposée au fond du flacon fut additionnée d'eau stérilisée, puis secouée et soumise à la centrifugation dans des tubes stérilisés. L'eau fut alors décantée et remplacée par

de l'eau nouvelle, après quoi on soumit encore le liquide à la centrifugation. On répéta ces manipulations jusqu'à ce que l'eau ne donnât plus de réaction avec la liqueur Fehling. La levure fut alors délayée dans de l'eau, et l'on en ajouta une ou plusieurs gouttes à la liqueur nutritive avec laquelle l'expérience devait être effectuée. Immédiatement après l'ensemencement, je comptai les cellules contenues dans une unité de volume. Le procédé suivi pour la numération se trouve décrit dans mon ouvrage: «Die Gärungsorganismen in der Theorie und Praxis der Alkoholgärungsgewerbe», Stuttgart 1906. Sur chaque flacon on prélève pour chaque numération au moins cinq échantillons, et pour chacun de ceux-ci on compte vingt carrés. La moyenne se trouve indiquée dans les tableaux ci-dessous. Pour la numération j'employais l'oculaire I et l'objectif V de Seibert, à l'exception des essais sur les Debaryomyces globosus et Schwanniomyces occidentalis, où je me suis servi de l'objectif III. Dans l'hématimètre de Zeiss, la goutte soumise à la numération est épaisse de $0^{mm},2$; la longueur du côté du grand carré de l'oculaire à réseau est avec l'oculaire V de $0^{mm},125$, et avec l'oculaire III $0^{mm},36$. Dans le premier cas, la quantité de liquide qui fait l'objet de la numération est, par conséquent, de 0,003125 mm^3, et dans le second cas de 0,02592 mm^3. Si donc on compte 10 cellules dans un grand carré, il y a, respectivement, 3200 et 385 cellules dans 1 mm^3, et l'on peut ainsi trouver le nombre réel de cellules dans 1 mm^3 en multipliant le nombre compté de cellules par 320 ou par 38,5.

Comme milieux nutritifs j'employais de l'eau de levure et une dissolution de sels dans l'eau distillée additionnée soit d'asparagine ou de peptone. La proportion de sucre était de 10 ou 5 pour cent, dans des cas exceptionnels seulement de 1 p. c. Voici la composition de la liqueur nutritive artificielle: Eau distillée 1000 cm^3, sulfate de magnésium $0^g,25$, phosphate de potassium primaire 5 g, asparagine ou peptone 5 g. A moins d'indication contraire, les sucres employés étaient ceux du commerce désignés comme étant «les plus purs»; il a été constaté pourtant par la suite qu'ils n'étaient pas tous absolument purs. J'ai cependant utilisé aussi des sucres recristallisés dans l'alcool et, en ce qui concerne la maltose, je lui ai fait subir dans certains cas une purification ultérieure en ajoutant préalablement à la solution une levure qui ne fermenterait que la dextrose et qui, par conséquent,

éliminerait toute trace de cette dernière, s'il y en avait. Il va sans dire qu'après ce traitement j'ai stérilisé à nouveau la solution.

Tous les liquides furent stérilisés par grandes portions qui n'avaient reçu aucune addition de sucre; ensuite on y mélangea des solutions de sucre dans l'eau, solutions qui avaient également été stérilisées au préalable; enfin on distribua les liquides dans de petits flacons. Cette manière d'agir présente cet avantage, qu'on a exactement le même liquide nutritif dans toutes les expériences et que les résultats sont comparables, ce qui est de la plus grande importance pour des expériences comme celles qui nous occupent.

Afin de débarrasser de ses éléments azotés l'air qui vient en contact avec le liquide nutritif, j'ai dans plusieurs de mes essais fait passer cet air à travers de l'acide sulfurique concentré et une dissolution d'hydroxyde de potassium; mais néanmoins il y a des points où l'on rencontre des matières azotées qu'on ne peut pas éviter. C'est ainsi que l'eau distillée se trouva contenir 0g,15 d'azote par litre; le phosphate de potassium primaire renfermait 0,06 ‰, la lactose la plus pure 0,025 ‰, la lactose pure du commerce 0,191 ‰, la maltose 0,14 ‰ et la maltose recristallisée 0,02 ‰. Ces doses minimes sont suffisantes pour qu'une multiplication puisse se produire, laquelle se manifeste par ma méthode de numération, et qui rend ces recherches si difficiles. Au surplus, la faible quantité de matière nutritive contenue dans l'intérieur des cellules au moment de l'ensemencement peut, elle aussi, donner lieu à une légère multiplication.

Tous les essais ont été effectués, sauf indication contraire, à l'obscurité et à 25 degrés centigrades.

Dans les tableaux ci-dessous sont employées les abréviations suivantes:

G = Eau de levure
D = Dextrose
L = Lactose
M = Maltose
S = Saccharose
K = Liquide nutritif artificiel (solution de sels)
A = Liquide nutritif artificiel additionné d'asparagine
P = — — — — de peptone
o = recristallisé.
r = purifié par fermentation

F = Récolte ou multiplication, c'est-à-dire le rapport du nombre de cellules trouvé au bout d'un certain temps dans une unité de volume (*b*), au nombre de cellules semées primitivement dans l'unité de volume (*a*), donc $F = \frac{b}{a}$.

Les nombres mis en regard des abréviations formant la première colonne représentent le nombre de cellules ensemencées dans l'unité de volume.

Saccharomyces cerevisiæ Hansen

EXPÉRIENCE II

La levure ensemencée avait été engendrée dans du moût de bière houblonné.

Ensemencé		Récolté au bout de 4 jours
DG	12.96	F = 14.03
LG	12.60	F = 3.41
MG	12.60	F = 21.57
SG	12.50	F = 23.96
G	12.50	F = 4.09

Ainsi donc, D, M et S ont été assimilés, tandis que L ne l'a pas été.

EXPÉRIENCE XV

Ensemencé		Au bout de 5 jours	Au bout de 10 jours
DA	7.82	F = 2.66	F = 3.95
LA	7.32	F = 1.30	
MA	7.82	F = 2.68	F = 6.25
SA	7.50	F = 3.66	F = 4.28
A	7.72	F = 1.18	

Cette expérience, à son tour, montre nettement que D, M et S ont été assimilés, et que L ne l'est pas. L'asparagine est ici une mauvaise source d'azote.

EXPÉRIENCE XVII

Ensemencé		Au bout de 3 jours	Au bout de 10 jours
DP	7.88	F = 12.18	F = 22.85
LP	7.40	F = 2.26	F = 3.23
MP	7.50	F = 18.24	F = 41.07
SP	7.72	F = 16.42	F = 37.05
P	7.98	F = 1.74	F = 1.85

D, M et S sont assimilés; L est douteux. La titration de LP avant le commencement et après la fin de l'expérience n'a pu fait constater de différence dans la quantité de sucre. Par conséquent, la peptone est ici une bonne source d'azote. En faisant l'épreuve de cette espèce de levure dans des flacons Freudenreich, suivant la méthode de Lindner & Saito, il fut constaté que DP, MP et SP avaient donné un développement abondant, qui paraissait être pareil dans ces trois cas; dans LP et P, le développement était extrêmement faible. Dans un essai ultérieur fait dans les mêmes conditions, LP montra un développement environ deux fois plus fort que celui de P, fait qui semble démontrer que L est susceptible d'être assimilé lorsque P sert de source d'azote.

Lindner & Saito ont trouvé: DA 2, LA ÷, MA 2 et SA 2, résultat qui concorde avec celui que j'ai obtenu.

Sacch. Carlsbergensis Hansen

EXPÉRIENCE III

Levure d'ensemencement produite dans du moût houblonné.

Ensemencé	Au bout d'un jour	Au bout de 2 jours
DG 15.00	F = 17.80	F = 33.63
LG 15.07	F = 1.37	F = 2.83
MG 14.92	F = 12.62	F = 27.02
SG 15.25	F = 17.45	F = 32.04
G 15.15	F = 1.47	F = 2.78

On voit que cette espèce s'assimile D, M et S, mais non L.

EXPÉRIENCE XIII

Ensemencé	Au bout d'un jour	Au bout de 2 jours	Au bout de 10 jours
DA 9.86	F = 6.52	F = 12.20	F = 17,57
LA 9.74	F = 1.15	F = 1.20	F = 2.23
MA 9.86	F = 6.65	F = 13.36	F = 33.94
SA 9.84	F = 5.75	F = 16.87	F = 17.75
A 9.84	F = 1.10	F = 1.19	F = 2.25

EXPÉRIENCE XVIII a

Ensemencé	Au bout d'un jour	Au bout de 2 jours	Au bout de 10 jours
DA 9.04	F = 7.69	F = 12.88	F = 13.40
LA 9.02	F = 1.65	F = 1.59	F = 1.69
MA 9.16	F = 5.67	F = 13.00	F = 26.65
SA 9.18	F = 5.66	F = 7.98	F = 20.03
A 9.08	F = 1.30	F = 1.27	F = 1.32

D, M et S sont donc assimilés, mais non L. Il paraît que l'asparagine est ici une assez bonne source d'azote.

Suivant la méthode de Lindner & Saito, j'ai trouvé: DA 1, LA ?, MA 3 et SA 2; dans un second essai: DA, MA et SA 3 (tous pareils), LA 1/4. Un troisième essai me donna DA 2, LA 1/2, MA 3, SA 1 et A ÷. Lindner & Saito ont DA 1, LA ?, MA 3 et SA 1.

La titration de LA (par la méthode de Bang) n'a pas fait constater de diminution dans la proportion de sucre.

EXPÉRIENCE XVIII b

Ensemencé	Au bout d'un jour	Au bout de 2 jours	Au bout de 10 jours
DP 9.18	F = 5.33	F = 12.03	F = 14.94
LP 9.42			F = 1.31
MP 9.74	F = 8.04	F = 13.55	F = 17.49
SP 9.34	F = 7.40	F = 11.29	F = 20.63
P 9.26		F = 1.22	F = 1.26

D, M et S sont assimilés, mais non L. Titré au bout de dix jours, LP ne montra pas de diminution du sucre. Suivant la méthode de Lindner & Saito, j'ai trouvé DP 3, LP 1/2, MP 3, SP 3 et P 1/4.

Sacch. ellipsoideus Hansen

EXPÉRIENCE IV

Ensemencé	Au bout d'un jour	Au bout de 2 jours
DG 18.12	F = 29.07	F = 34.67
LG 17.96	F = 2.39	F = 3.35
MG 18.36	F = 28.37	F = 38.95
SG 18.12	F = 34.46	F = 39.56
G 18.10	F = 2.34	F = 3.31

D, M et S sont assimilés, mais non L.

EXPÉRIENCE XII

Ensemencé	Au bout d'un jour	Au bout de 2 jours	Au bout de 10 jours
DA 7.94	F = 9.47	F = 11.51	F = 43.42
LA 7.24	F = 1.49	F = 1.51	F = 1.79
MA 7.40	F = 13.54	F = 34.36	F = 52.27
SA 7.12	F = 10.17	F = 24.74	F = 22.87
A 7.66	F = 1.24	F = 1.23	F = 1.44

D, M et S sont assimilés, mais non L. Suivant Lindner & Saito, j'ai trouvé: DA 2, LA ½, MA 3, SA 1 et A ÷. Lindner & Saito ont: DA 2, LA ?, MA 3 et SA 2.

EXPÉRIENCE XXXII

Les sucres expérimentés avaient été recristallisés, et l'air des flacons avait été débarrassé de ses matières azotées. Cette expérience était effectuée à la température ordinaire du laboratoire.

Ensemencé	Au bout de 4 jours	Au bout de 10 jours
oDA 9.20	F = 24.92	F = 29.62
oMA 9.56	F = 40.74	F = 50.06
oSA 9.28	F = 12.96	F = 29.89
oDK 9.78	F = 1.82	
oMK 9.58	F = 1.78	
oSK 9.90	F = 1.54	

Cette expérience montre à l'évidence que l'asparagine est une bonne source d'azote pour cette espèce; en outre, que M, D et S sont assimilés, D et S également bien, pourtant D plus rapidement que S.

Sacch. Pastorianus Hansen

EXPÉRIENCE XVI

Ensemencé	Au bout de 2 jours	Au bout de 5 jours	Au bout de 10 jours
DG 16.00	F = 25.82	F = 27.09	F = 34.46
LG 16.24	F = 2.92	F = 3.64	F = 4.05
MG 15.70	F = 21.92	F = 23.18	F = 32.87
SG 15.72	F = 24.34	F = 26.41	F = 37.57
G 15.86	F = 2.85	F = 3.57	F = 3.95
DA 15.78	F = 12.96	F = 20.29	F = 19.45
LA 15.94	F = 1.59		F = 3.12
MA 16.24	F = 13.46	F = 23.94	F = 33.91
SA 16.02	F = 8.74	F = 9.05	F = 19.59
A 16.06	F = 1.46		F = 2.20

D, M et S sont donc assimilés, mais non L.

Suivant Lindner & Saito, j'ai trouvé: DA 3, LA ?, MA 4, SA 2 (3) et A ÷. Ils ont: DA (÷) 1, LA ?, MA 3 et SA ?. Comme on le voit, ce résultat ne concorde nullement avec celui obtenu par moi. F, pour ce qui concerne M, est pareil dans MG et M, tandis que pour D et S il est notablement plus élevé dans DG et SG que dans DA et SA.

Sacch. turbidans Hansen

EXPÉRIENCE I

Ensemencé	Au bout d'un jour	Au bout de 2 jours
DG 16.09	F = 21.21	F = 42.57
LG 16.72	F = 2.33	F = 3.38
MG 15.90	F = 18.70	F = 28.24
SG 16.59	F = 25.25	F = 53.49

Ainsi, D, M et S ont bien subi l'assimilation. Quoiqu'il n'ait pas été fait d'ensemencement dans G, ce qui eût permis une comparaison avec le résultat obtenu dans LG, je pense que, étant donné ce qu'ont appris les expériences sur les espèces précédentes, il est légitime d'admettre que L n'est pas assimilé. Ce qui frappe surtout dans cette expérience-ci, c'est la multiplication abondante produite dans S où, en effet, elle est à peu près deux fois aussi forte que dans M.

Je n'ai pas fait d'ensemencement avec l'asparagine comme source d'azote.

Lindner & Saito trouvèrent: DA 1, LA ?, MA 3 et SA 2.

Sacch. Marxianus Hansen

Cette espèce ne pouvant pas faire fermenter la maltose, il y avait un intérêt tout particulier à connaître son pouvoir assimilateur vis-à-vis de ce sucre.

EXPÉRIENCE V

Ensemencé	Au bout d'un jour	Au bout de 2 jours
DG 22.22	F = 29.71	F = 38.38
LG 22.14	F = 3.37	F = 6.59
MG 22.08	F = 5.81	F = 11.80
SG 22.38	F = 32.01	F = 32.65
G 22.38	F = 2.57	F = 5.29

Il résulte de cette expérience que D, M et S sont bien assimilables, tandis que L l'est à peine.

EXPÉRIENCE VII

Ensemencé		Au bout d'un jour	Au bout de 2 jours
DA	10.14	F = 8.55	F = 10.36
LA	9.42	F = 3.78	F = 3.85
MA	9.70	F = 8.33	F = 8.91
SA	9.22	F = 9.85	F = 15.17
A	9.66	F = 1.03	F = 1.01

D, M et S s'assimilent, L à peine.

EXPÉRIENCE XX

Ensemencé		Au bout d'un jour	Au bout de 2 jours	Au bout de 10 jours
DA	10.76	F = 14.04	F = 16.25	F = 24.25
LA	10.82	F = 2.51	F = 3.19	F = 10.09
MA	10.36	F = 6.96	F = 7.54	F = 13.34
SA	10.80	F = 12.92	F = 14.08	F = 24.38
A	10.26	F = 1.92	F = 1.96	F = 4.33
DP	10.56	F = 37.96	F = 48.76	F = 70.89
LP	10.48	F = 4.46	F = 7.67	F = 21.96
MP	10.74	F = 6.87	F = 7.97	F = 11.86
SP	10.80	F = 36.08	F = 50.78	F = 77.40
P	10.92	F = 2.37	F = 2.40	F = 3.27

Ainsi, il paraît que la peptone constitue pour cette espèce une source d'azote beaucoup meilleure que l'asparagine. La multiplication observée après dix jours dans DP et SP est bien plus forte que d'ordinaire.

Pour autant qu'on peut le voir par cette expérience, les quatre sucres expérimentés s'assimilent tous. MP, MA, LP et LA ont été analysés au point de vue d'une teneur possible en alcool: ils s'en sont montrés exempts; par conséquent, ces deux sucres, maltose et lactose, ont été purs de dextrose et de tout autre sucre fermentescible. Suivant la méthode de Lindner & Saito, j'ai trouvé: DA 1, LA 1/8, MA 2, SA 1 et A ÷; de plus, DP 3, LP 3, MP 3—2, SP 3 et P ÷.

Lindner et Saito ont: DA 1, LA ÷, MA 1, SA 1; résultats qui ne concordent point avec les miens.

EXPÉRIENCE XXI

Une partie de la maltose sur laquelle portait cette expérience, avait été traitée préalablement par une levure capable de faire fermenter D, afin de la débarrasser d'une teneur possible en D (rM); l'autre partie était de la maltose ordinaire.

Ensemencé		Au bout de 5 jours
rMG	4.98	F = 13.58
G	4.98	F = 9.50
MK	5.46	F = 8.70

Le titrage au moyen de la liqueur de Bang fit constater qu'une partie de la maltose purifiée avait disparu et doit, en conséquence, avoir été assimilée.

La méthode de Lindner & Saito me donna au bout de dix jours: rMG 2—3, G 2—3 et MK 1—2. Il semble, par conséquent, que rMG et G se soient ici comportés pareillement, d'où l'on pourrait conclure qu'il n'y aurait point d'assimilation. C'est une preuve que la méthode Lindner & Saito ne peut pas servir lorsqu'il est question d'une assimilation légère.

Dans MK — qui était dénué de toute source d'azote, abstraction faite de la minime proportion contenue dans l'eau et dans le phosphate potassique primaire —, la quantité de M s'est trouvée la même après dix jours qu'au début de l'expérience; M avait donc entièrement échappé à l'assimilation.

EXPÉRIENCE XXII

J'ai opéré en partie sur la maltose ordinaire, en partie sur de la maltose recristallisée contenant 0,02 ‰ d'azote.

Ensemencé		Au bout de 3 jours	Au bout de 5 jours	Au bout de 10 jours
MK	16.88	F = 4.19	F = 4.21	F = 4.43
oMK	16.64	F = 3.30	F = 3.44	F = 3.48
oMA	16.50	F = 3.72	F = 3.82	F = 6.06
A	16.24	F = 2.66	F = 3.40	F = 5.76

De ces résultats il ressort que la multiplication était plus grande dans le liquide nutritif contenant M que dans celui renfermant oM, et plus grande dans oMA que dans A, ce qui montre qu'une certaine quantité de M a été assimilée.

Suivant la méthode de Lindner & Saito, j'ai trouvé: MK 1, oMK 1, oMA 1 et A ?.

EXPÉRIENCE XXIV

Dans celle-ci il fut également employé de la maltose recristallisée contenant 0,02 ‰ d'azote. On fit passer de l'air sur le liquide renfermé dans les flacons de culture, auquel on avait au préalable enlevé ses matières azotées. L'expérience était faite à la température ordinaire du laboratoire.

	Ensemencé	Au bout de 5 jours	Au bout de 10 jours
oMA	10.52	F = 5.14	F = 7.42
A	9.86	F = 1.16	F = 1.82

Comme il n'est pas probable que la multiplication constatée dans oMA soit due à l'infime dose d'azote de M, il paraît évident qu'une certaine quantité de M a subi l'assimilation.

EXPÉRIENCE XXVII

Les sucres employés avaient été recristallisés, et l'air avait été débarrassé de ses matières azotées. Température ordinaire du laboratoire.

	Ensemencé	Au bout de 2 jours	Au bout de 5 jours	Au bout de 10 jours
oDA	10.92	F = 15.59	F = 25.97	F = 28.57
oMA	10.66	F = 3.14	F = 3.74	F = 3.81
oSA	10.70	F = 17.51	F = 17.81	F = 17.72

Il est manifeste que D et S ont été assimilés; mais on ne sait comment s'expliquer que dans oMA la multiplication n'est que moitié aussi grande que dans l'expérience précédente, attendu que le liquide nutritif et la quantité ensemencée sont identiquement les mêmes.

EXPÉRIENCE XXXIV

J'ai employé du liquide nutritif sans des matières azotées et additionné de 1 % de oM. Il fut distribué sur deux flacons, dont l'un, *b*, reçut un apport d'air débarrassé de matières azotées, tandis que dans l'autre, *c*, on fit arriver de l'air ordinaire. Cette expérience avait le but de rechercher si l'espèce dont il s'agit peut s'assimiler l'azote libre de l'air. Par *a* nous désignons un ballon servant à la détermination de la teneur en azote immédiatement après l'ensemencement.

Ensemencé	Après 11 jours
a 12.10	
b 12.14	Nombre de cellules 11.83
c 12.44	— · — 13.14

Dans 75 cm³ de *a*, je trouvai 0mg,216 d'azote; au bout de onze jours, dans *b*: 0mg,205 d'azote, dans *c*: 0mg,21 d'azote. Ainsi, l'assimilation d'azote libre était nulle.

Sacch. fragilis Jørgensen

EXPÉRIENCE VI

Ensemencé	Au bout d'un jour	Au bout de 2 jours
DG 79.23	F = 14.84	F = 14.58
LG 76.16	F = 12.44	F = 14.12
MG 74.76	F = 2.18	F = 3.34
SG 74.16	F = 14.09	F = 15.50
G 77.84	F = 1.73	F = 2.17
DA 78.51	F = 1.31	F = 1.70
LA 75.95	F = 1.40	F = 1.32
MA 74.35	F = 1.89	F = 1.95
SA 78.40	F = 1.38	F = 2.09
A 74.10	F = 1.23	F = 1.23

Comme les cellules de cette espèce sont très petites, la quantité ensemencée fut un peu plus grande que dans les autres expériences. Elle fait fermenter la dextrose et la lactose, mais non la maltose ni la saccharose. L'essai fait dans G démontre qu'elle s'assimile D, L et S, mais à peine M; de celui dans A il ne se dégage aucune conclusion, si ce n'est que l'asparagine ne vaut rien comme source d'azote. G, au contraire, semble en constituer une excellente.

Zygosaccharomyces Priorianus Kløcker

Les cellules de cette espèce, ayant une tendance marquée à se pelotonner, sont difficiles à compter.

EXPÉRIENCE XIV a

Ensemencé	Au bout de 2 jours	Au bout de 5 jours	Au bout de 10 jours
DG 15.66	F = 13.24		F = 21.38
LG 16.28	F = 3.42	F = 4.52	F = 8.80
MG 17.78	F = 13.40	F = 35.84	F = 38.49
SG 17.36	F = 4.57	F = 6.41	F = 16.59
G 16.50	F = 2.07		F = 2.31

Il est hors de doute que D, M et S sont assimilés; L paraît l'être aussi, vu que dans LG la multiplication est à peu près quatre fois plus grande que dans G.

EXPERIENCE XIV b

Ensemencé	Au bout de 2 jours	Au bout de 5 jours	Au bout de 10 jours
DA 16.74	F = 11.16	F = 14.73	F = 17.80
LA 16.70	F = 1.77	F = 1.56	F = 1.70
MA 16.78	F = 9.29	F = 15.69	F = 20.21
SA 17.28	F = 5.18	F = 8.31	F = 17.46
A 17.36	F = 1.25	F = 1.31	F = 1.56

L'asparagine servant de source d'azote, il n'y a donc pas assimilation de L, mais bien de D, M et S. Ce résultat est absolument différent de celui obtenu par Lindner & Saito, à savoir: DA 1, LA ÷, MA ÷, SA ÷.

EXPÉRIENCE XXIX

Cette expérienee a porté sur des sucres recristallisés.

Ensemencé	Au bout de 5 jours	Au bout de 10 jours
oDA 10.06	F = 18.87	F = 37.01
oMA 9.86	F = 15.94	F = 40.74
oSA 9.90	F = 11.04	F = 19.07
oDK 10.08	F = 3.22	F = 3.98
oMK 9.94	F = 3.47	F = 4.08
oSK 10.02	F = 2.90	F = 3.06

Pour ce qui est de la multiplication dans A, nous voyons confirmé le résultat obtenu par l'expérience qui précède; l'essai où K a servi de liquide nutritif, ne permet de tirer aucune conclusion. On a quelque peine à s'expliquer comment il se fait que A donne ici une multiplication notablement plus grande que dans l'expérience précédente.

EXPÉRIENCE XXXV

Comme précédemment, seulement avec cette différence qu'on a apporté aux flacons de l'air débarrassé de matières azotées. Température ordinaire du laboratoire.

Ensemencé		Au bout de 4 jours	Au bout de 9 jours
oDA	9.56	F = 12.72	F = 15.48
oMA	10.22	F = 3.86	F = 10.63
oSA	9.76	F = 9.03	F = 13.49
oDK	9.82	F = 3.23	F = 4.35
oMK	10.54	F = 3.02	F = 4.25
oSK	10.18	F = 3.02	F = 3.82

On constate encore ici que D, M et S sont assimilés dans A.

Saccharomycodes Ludwigii Hansen

EXPÉRIENCE VIII

Ensemencé		Au bout de 2 jours	Au bout de 3 jours
DG	11.36	F = 21.86	F = 22.52
LG	11.00		F = 1.38
MG	11.10	F = 2.31	F = 2.93
SG	11.16	F = 21.71	F = 23.12
G	11.18		F = 1.45
DA	11.72	F = 5.55	F = 5.86
LA	11.08	F = 2.00	F = 2.00
MA	10.78	F = 2.32	F = 2.49
SA	11.30	F = 5.15	F = 5.08
A	11.20	F = 1.86	

L'asparagine est donc une mauvaise source d'azote pour cette espèce. D et S sont assimilés; quant à M, c'est douteux en ce qui concerne G; mais il est certain que L n'est assimilé ni dans G ni dans A.

EXPÉRIENCE XIX

	Ensemencé	Au bout de 2 jours	Au bout de 3 jours	Au bout de 10 jours
DA	10.88	F = 3.27	F = 4.12	F = 7.50
LA	11.08	F = 1.06	F = 1.02	F = 1.22
MA	10.92	F = 2.49	F = 2.58	F = 2.73
SA	11.54	F = 4.35	F = 4.21	F = 6.92
A	11.04	F = 1.01		
DP	10.88	F = 7.37	F = 7.42	
LP	11.72	F = 1.03		
MP	11.28	F = 2.24		
SP	11.08	F = 6.78	F = 7.63	F = 11.52
P	11.18	F = 1.07		

Quoique, par suite d'un petit accident survenu, l'essai sur P soit un peu défectif, on voit que la peptone est une meilleure source d'azote que l'asparagine. Ici encore, D et S se montrent assimilables. Mais pour ce qui concerne M, il est douteux s'il l'est. C'est d'autant plus étonnant que d'après Lindner & Saito cette espèce ne croît que dans M, assez médiocrement il est vrai. Ces mêmes auteurs trouvèrent DA ÷, LA ÷, MA 1, et SA ÷, résultat auquel je suis aussi arrivé en procédant suivant leur méthode qui, en effet, me donna: DA ÷?, LA ÷?, MA 1 et SA ÷?; enfin, A ÷. Il y a donc une discordance entre ce résultat et celui obtenu par numération. En outre, la méthode Lindner & Saito me donna: DP 2, LP 1/4, MP 1, SP 2—3 et P 1/4.

Ainsi donc, il est démontré encore une fois que leur méthode n'est pas applicable.

EXPÉRIENCE XXIII

Elle a porté sur des sucres soit ordinaires, soit recristallisés.

	Ensemencé	Au bout de 5 jours	Au bout de 10 jours
DA	10.94	F = 4.03	F = 5.07
MA	10.40	F = 3.29	F = 3.61
SA	10.56	F = 3.49	F = 4.41
oDA	10.36	F = 3.32	F = 3.55
oMA	10.42	F = 2.84	F = 2.96
oSA	10.22	F = 3.48	F = 3.89

Ici aussi, la multiplication est le moins avançée dans M; mais l'ensemencement de la même levure suivant le procédé de Lindner & Saito me donna: DA ÷ ?, MA 2, SA ÷ ?, oDA ÷ ?,

oMA 2 (ou peut-être un peu moins), oSA ÷ ?. Au bout de trois mois, même résultat. Celui-ci est donc sensiblement identique à celui de l'expérience précédente.

EXPÉRIENCE XXV

L'air apporté aux flacons avait été débarrassé des matières azotées. Température ordinaire du laboratoire.

	Ensemencé	Au bout de 3 jours	Au bout de 5 jours	Au bout de 10 jours
oSA	9.72	F = 5.94	F = 6.37	F = 6.11
A	9.88	F = 1.15		F = 1.34

EXPÉRIENCE XXVI

	Ensemencé	Au bout d'un jour	Au bout de 5 jours	Au bout de 10 jours
oDA	9.72	F = 3.80	F = 4.49	F = 5.81
oMA	10.18	F = 1.56	F = 1.77	F = 2.83
oSA	9.74	F = 3.74	F = 4.85	F = 6.00
A	10.26	F = 1.26	F = 1.21	F = 1.45

Ces deux expériences viennent encore confirmer le précédent résultat. Vu que dans oSA la multiplication est pareille que l'air ait été dépourvu de ses matières azotées ou non, il semblerait que S. Ludwigii n'absorbe pas de ces matières venant de l'air. Il est à remarquer que les deux expériences portaient sur une seule et même levure.

EXPÉRIENCE XXXIII

Le but de celle-ci était de rechercher si le S. Ludwigii a la faculté de s'assimiler de l'azote libre de l'air. Le milieu de culture était K + 1 % de oM. Après ensemencement, le liquide nutritif fut distribué dans trois ballons Pasteur, 100 cm³ dans chacun. Température ordinaire du laboratoire. Dans le premier ballon *a*, on a dosé l'azote aussitôt après l'ensemencement. *b* a reçu de l'air dépourvu des matières azotées, *c*, de l'air ordinaire.

	Ensemencé	Après 11 jours
a	10.62	
b	10.96	F = 1.39
c	10.88	F = 1.68

Teneur en azote trouvée:

Dans 75 cm³ de *a* 0mg,523
— 75 — · *b* après 11 jours 0mg,476
— 75 — · *c* — 11 — 0mg,484

Il n'a donc pas été assimilé d'azote libre de l'air.

Debaryomyces globosus Kløcker

EXPÉRIENCE X

	Ensemencé	Au bout de 3 jours	Au bout de 10 jours
DG	11.62	F = 56.22	F = 56.80
LG	11.14	F = 1.72	F = 3.33
MG	11.50	F = 5.20	F = 6.67
SG	11.20	F = 33.69	F = 55.03
G	10.80	F = 1.90	F = 3.17
DA	10.64	F = 65.51	F = 95.39
LA	10.40	F = 1.27	F = 1.80
MA	10.74	F = 3.23	F = 4.74
SA	10.94	F = 55.12	F = 63.73
A	11.22	F = 1.16	F = 1.34

Il ressort de ce tableau que le Debaryomyces s'assimile D et S; l'assimilation de M paraît douteuse et, en tout cas, minime. L'asparagine est pour D et S une meilleure source d'azote que G. Quant à L, il est manifeste qu'il n'est pas assimilé, contrairement à l'indication de Linder & Saito, qui trouvèrent: DA 3, LA 1, MA 2—3 et SA 1. En appliquant leur méthode, j'ai trouvé: DA 4, LA ÷. MA 2, SA 3 et A ÷, résultat qui, on le voit, concorde à peu de chose près avec celui de la numération.

Cette espèce fait fermenter D et S, mais non L ni M.

Schwanniomyces occidentalis Kløcker

EXPÉRIENCE XI a

	Ensemencé	Au bout de 3 jours	Au bout de 5 jours	Au bout de 10 jours
DG	7.62	F = 22.26	F = 33.35	F = 54.22
LG	7.76	F = 4.51	F = 8.99	F = 13.90
MG	7.22	F = 6.93	F = 17.31	F = 49.39
SG	7.54	F = 18.44	F = 27.70	F = 52.86
G	8.50	F = 5.18	F = 8.65	F = 13.18

Ainsi, D, M et S sont assimilés, M un peu plus lentement que les deux autres.

EXPÉRIENCE XI b.

	Ensemencé	Au bout de 3 jours	Au bout de 5 jours	Au bout de 10 jours
DA	7.58	F = 16.93	F = 29.15	F = 39.34
LA	7.02	F = 6.38	F = 13.03	F = 21.81
MA	7.12	F = 16.48	F = 24.00	F = 43 27
SA	7.22	F = 20.74	F = 29.92	F = 31.85
A	7.24	F = 5.28	F = 11.51	F = 16.42

Ici encore, on voit s'assimiler D, M et S. Quant à L, on ne saurait conclure de façon certaine; il paraît pourtant probable qu'il s'assimile, bien que seulement en proportion minime. G semble constituer une meilleure source d'azote que l'asparagine, sauf en ce qui concerne L.

Lindner & Saito trouvèrent: DA 1, LA ?, MA 2, et SA 2. En employant leur méthode, j'ai trouvé: DA 3, LA 2, MA 4, SA 3 et A 1, résultat qui concorde avec celui de la numération, excepté pour ce qui regarde L. L'espèce fait fermenter D et S, mais non L ni M.

Schizosaccharomyces octosporus Beyerinck

La numération est très difficile pour cette espèce, parce que ses cellules ont une tendance à se pelotonner.

EXPÉRIENCE IX a

	Ensemencé	Au bout de 2 jours	Au bout de 10 jours
DG	12.30	F = 10.67	F = 17.48
LG	13.58	F = 1.08	F = 1.39
MG	13.72	F = 6.65	F = 12.03
SG	12.84	F = 1.08	F = 4.58
G	12.84	F = 1.08	

Il appert de ce tableau que D et M sont bien assimilés. L'espèce en expérience ne peut pas faire fermenter S; néanmoins, une faible proportion de ce sucre semble être assimilée. Par suite d'un accident — le flacon s'étant brisé —, la numération ne put pas au bout de dix jours être appliquée à G. Vu cependant que la multiplication est bien plus forte en SG qu'en LG, il y a lieu d'admettre qu'une assimilation s'est produite. Lindner (4) dit, il est vrai, que L peut exercer une action en quelque sorte vénéneuse, parce que dans une dissolution d'asparagine exempte de L il vit le Sacch. coreanus se développer avec une intensité deux fois aussi grande que dans une solution pareille additionnée de L.

EXPÉRIENCE IX b

	Ensemencé	Au bout de 2 jours	Au bout de 10 jours
DA	12.44	F = 1.23	F = 1.67
LA	12.28	F = 1.19	
MA	12.62	F = 1.29	F = 2.82
SA	12.18	F = 1.20	F = 1.28
A	11.76	F = 1.14	

Cette expérience montre avec évidence que l'asparagine est une source d'azote très mauvaise pour l'espèce concernée. Peut-être une faible proportion de M a-t-elle été assimilée. Selon Lindner & Saito, cette espèce ne s'assimile aucun sucre; je suis arrivé au même résultat en appliquant leur méthode aux quatre sucres dont il s'agit. L'ensemencement en chambres humides ne m'a pas donné de développement non plus. La cause en est vraisemblement dans la quantité ensemencée excessivement minime. Beyerinck (2) trouva que l'espèce assimilait D et M, mais non S. Elle fait fermenter D et M, mais pas L et S.

Les expériences relatives à cette espèce démontrent avec une évidence toute particulière qu'il ne suffit nullement d'affirmer que telle ou telle espèce de levure peut s'assimiler tel ou tel sucre, sans indiquer en même temps la source d'azote qu'on aura utilisée.

3. Coup d'œil rétrospectif

Les recherches que nous venons de relater portent sur ces douze espèces de levure: Saccharomyces cerevisiæ Hansen, S. Carlsbergensis Hansen, S. ellipsoideus Hansen, S. Pastorianus Hansen, S. turbidans Hansen, S. Marxianus Hansen, S. fragilis Jørgensen, Zygosaccharomyces Priorianus Kløcker, Saccharomycodes Ludwigii Hansen, Debaryomyces globosus Kløcker, Schwanniomyces occidentalis Kløcker et Schizosaccharomyces octosporus Beyerinck. Les milieux de culture étaient: 1° Eau de levure, 2° une solution aqueuse de sulfate de magnésium et de phosphate de potassium primaire, additionnée d'asparagine, et 3° la même liqueur, sauf que l'asparagine était remplacée par la peptone. Les sucres mis en expérience étaient: dextrose, lactose, maltose et saccharose, ajoutés à la dose de 10 ou 5 p. c., par exception seulement 1 p. c. La multiplication des levures fut déterminée par numération. Les abréviations employées ci-dessous se trouvent expliquées à la page 15.

S. cerevisiæ

Avec G comme source d'azote, la multiplication était forte au bout de quatre jours, surtout dans SG, puis dans MG, et enfin dans DG. Avec l'asparagine comme source d'azote, elle était minime après dix jours; le plus grande dans MA, puis vient SA, et ensuite DA. Lorsque la peptone servait de source d'azote, on

observait après dix jours une multiplication très énergique, particulièrement dans MP; elle était un peu moins forte dans SP et moindre encore dans DP; dans LP elle était douteuse.

D, M et S ont été assimilés dans tous les cas; L l'est peut-être dans LP, mais en tout cas très peu.

L'asparagine s'est trouvée être une très mauvaise source d'azote. L'eau de levure, par contre, en est une assez bonne; la peptone de même.

S. Carlsbergensis

La multiplication avec G comme source d'azote, au bout de deux jours, était intense, surtout en DG et au même degré en SG; en MG elle était un peu moindre. Avec l'asparagine comme source d'azote, elle était assez forte, le plus en MA, puis en SA, ensuite en DA (dans un cas elle était pourtant aussi forte en DA qu'en SA). Lorsque la peptone servait de source d'azote, elle était moindre; par ordre décroissant: SP, MP, DP.

Dans tous les cas, il y avait assimilation de D, M et S, mais non de L.

L'asparagine s'est révélée être une bonne source d'azote, presque égalant l'eau de levure et la peptone.

S. ellipsoideus

La multiplication avec G comme source d'azote, après deux jours, était intense, principalement en SG, un peu moins en MG et encore un peu moins en DG. Avec l'asparagine comme source d'azote, après dix jours, multiplication intense en MA, légèrement moindre en DA; en SA, moitié aussi forte qu'en DA. L'emploi de sucres recristallisés donna, au bout de dix jours, la plus forte multiplication en MA; en DA et, au même degré, en SA, elle était sensiblement moindre. La peptone n'a pas été employée dans les expériences cur cette espèce.

Dans tous les cas, D, M et S ont été assimilés, mais non L.

L'asparagine et l'eau de levure constituent de bonnes sources d'azote.

S. Pastorianus

Au bout de dix jours, on constata avec G comme source d'azote, une multiplication intense, particulièrement en SG; elle l'était un peu moins en DG, et encore un peu moins en MG. Avec l'asparagine comme source d'azote, après le même laps de

temps, elle était aussi forte en MA qu'en MG, notablement moindre en DA et de même en SA. La peptone n'était pas employée dans les essais sur cette espèce.

Dans tous les cas, il y a eu assimilation de D, M et S, mais non de L.

L'asparagine est une assez bonne source d'azote; toutefois, pour D et S elle est notablement inférieure à l'eau de levure.

S. turbidans

L'emploi de G comme source d'azote donna, au bout de deux jours, une multiplication forte, surtout en SG; en DG elle était lègèrement moindre, en MG relativement réduite. Aucune expérience ne fut faite avec d'autres sources d'azote.

Dans tous les cas, D, M et S ont subi l'assimilation, mais L non.

L'eau de levure constitue une bonne source d'azote.

S. Marxianus

La multiplication constatée au bout de deux jours, G. servant de source d'azote, était forte, surtout en DG, légèrement moindre en SG, encore moindre en MG. Avec l'asparagine comme source d'azote, elle se montra, également après deux jours, assez forte, particulièrement en SA; en DA elle l'était un peu moins, en MA encore un peu moins. LA accusait une multiplication trois fois plus grande que A. Dans une autre expérience, elle était au bout de dix jours à son maximum en SA aussi bien qu'en DA, un peu plus d'à moitié aussi grande en MA, et en LA légèrement moindre qu'en MA (environ deux fois et demie plus grande qu'en A). Avec la peptone comme source d'azote, il y avait après dix jours une multiplication fort intense, surtout en SP et — bien qu'à un degré un peu moindre — en DP; en LP elle était environ double de ce qu'elle était en MP, et environ sept fois plus forte qu'en P.

L'emploi de sucres recristallisés a donné la multiplication la plus intense en DA; en SA elle était un peu inférieure; et en MA beaucoup moindre, pourtant dans un cas environ quatre fois aussi grande qu'en A.

D, M et S ont été assimilés dans tous les cas et, lorsque la peptone servait de source d'azote, L l'a été aussi. Ce Saccharomyces ne peut faire fermenter ni M ni L.

Comme source d'azote la peptone est supérieure à l'eau de levure, qui elle-même l'emporte sur l'asparagine.

Quelques essais particuliers firent voir que cette espèce ne peut pas assimiler l'azote libre de l'air.

S. fragilis.

Dans le cas où G servait de source d'azote, la multiplication était après deux jours à peu près de même intensité en DG, LG et SG, tandis qu'en MG et également en G elle était très faible. L'emploi de l'asparagine comme source d'azote n'a donné, après deux jours, qu'une multiplication extrêmement minime dans tous les flacons.

Avec G comme source d'azote, il y a assimilation de D, L et S, mais pas de M. L'asparagine servant de source d'azote, il semble ne pas y avoir d'assimilation de sucre. Cette espèce fait fermenter D et L, mais pas M ni S.

L'eau de levure est une bonne source d'azote, contrairement à l'asparagine, qui ne peut pas servir du tout.

Zygos. Priorianus

L'emploi de G comme source d'azote a donné, au bout de dix jours, une multiplication intense, surtout en MG: en DG, elle était un peu moindre, et en SG un peu moindre encore. En LG, elle était environ à moitié aussi grande qu'en SG et environ quatre fois plus grande qu'en G. Avec l'asparagine comme source d'azote, en obtient au bout de dix jours une multiplication intense, surtout en MA; un peu moindre en DA et également en SA. En LA, la même multiplication extrêmement minime qu'en A. Les sucres recristallisés ont accusé également, au bout de dix jours, la multiplication la plus forte en MA, légèrement moindre en DA et notablement moindre en SA. La peptone n'a pas été employée comme source d'azote.

D, M et S ont été assimilés dans tous les cas. Lorsque G sert de source d'azote, il paraît probable que L subit à son tour l'assimilation, bien que seulement en faible proportion. Cette espèce ne fait pas fermenter ce dernier sucre.

Tant l'eau de levure que l'asparagine sont de bonnes sources d'azote.

S. Ludwigii

G employé comme source d'azote a donné, au bout de trois jours, la multiplication la plus forte en SG; en DG, elle était légèrement moindre. En MG, elle paraissait douteuse. Avec l'asparagine comme source d'azote, la multiplication au bout de trois et même de dix jours, était minime, de même grandeur en DA qu'en SA ; en LA et MA, elle était douteuse. La peptone comme source d'azote donna le même résultat; toutefois, la multiplication était un peu plus forte. Lorsqu'on emploie des sucres recristallisés, les différences de multiplication en DA, MA et SA après dix jours sont inférieures à celles constatées dans les expériences ci-dessus.

D et S sont assimilés dans tous les cas. Quant à M, il est fort douteux qu'il y ait assimilation; des essais pratiqués d'une autre manière portent à le croire; peut-être des matières azotées dans l'air et dans M ont-elles une influence particulière. L n'est pas assimilé. Le S. Ludwigii fait fermenter D et S, mais pas L et M.

L'eau de levure est une bonne source d'azote; la peptone et surtout l'asparagine en sont de mauvaises.

Des essais particuliers ont démontré que cette espèce ne s'assimile pas l'azote libre de l'air.

Debaryom. globosus

G employé comme source d'azote a donné au bout de dix jours une multiplication forte tant en DG qu'en SG. En MG elle était faible, bien que deux fois plus forte qu'en LG et G. Avec l'asparagine comme source d'azote, elle était après dix jours extrêmement forte en DA, forte en SA, très faible en MA, bien qu'environ trois fois plus forte qu'en LA et A. La peptone n'a pas été employée comme source d'azote.

D et S sont donc assimilés. Quant à M, c'est douteux; en tout cas, il ne l'est qu'en proportion minime. Des essais faits d'une autre manière semblent cependant prouver que M peut être assimilé. L n'est pas assimilé. Cette espèce fait fermenter D et S, mais pas L et M.

L'eau de levure est une bonne source d'azote, et l'asparagine l'est encore davantage.

Schwanniom. occidentalis

Lorsque G servait de source d'azote, la multiplication au bout de dix jours était le plus forte et de même grandeur en DG et SG, un peu moindre en MG; en LG aussi bien qu'en G, elle était assez forte. L'asparagine employée comme source d'azote a donné après dix jours la multiplication la plus forte en MA, un peu moins forte en DA, un peu moindre encore en SA. En LA, multiplication assez forte, d'environ 1/4 plus forte qu'en A. La peptone n'a pas été employée comme source d'azote.

D, M et S sont assimilés. L l'est probablement aussi, bien qu'en faible proportion seulemcnt; des essais faits d'une autre manière semblent le démontrer. Cette espèce fait fermenter D et S, mais pas L et M.

L'eau de levure aussi bien que l'asparagine sont de bonnes sources d'azote.

Schizos. octosporus

Avec G comme source d'azote, multiplication au bout de dix jours la plus forte en DG, légèrement moindre en MG, faible en SG. Avec l'asparagine, après dix jours multiplication à peu près nulle. La peptone n'a pas été essayée.

Il est certain que D et M sont assimilés; pour S, c'est douteux, bien que probable. L n'est pas assimilé. L'espèce fait fermenter D et M, mais pas L et S.

L'eau de levure est une assez bonne source d'azote; au contraire, l'asparagine ne s'y prête pas du tout.

4. Résumé

des principaux résultats

Il résulte de mes recherches ce qui suit:

Il est souvent bien difficile d'établir si une espèce de levure donnée s'assimile ou non tel ou tel sucre. C'est qu'il y a une foule de facteurs qui entrent en jeu, et qu'ils ne sauraient tous être éliminés, surtout lorsqu'il ne s'agit que d'une assimilation minime. Nous en avons parlé dans l'introduction.

Tant la multiplication que l'assimilation dépendent beaucoup des sources d'azote employées. Par conséquent, on ne peut affirmer d'une façon générale que telle levure a la faculté de s'assimiler tel sucre, sans indiquer en même temps la source d'azote dont

on s'est servi. L'asparagine est une bonne source d'azote pour certaines espèces, alors que pour d'autres elle en est une mauvaise ou tout à fait inutilisable.

Il peut arriver dans certaines conditions qu'une levure soit à même de s'assimiler un sucre qu'elle ne peut pas faire fermenter.

Dans la plupart des cas, l'assimilation ne saurait être déterminée avec précision que par une numération des cellules développées. C'est seulement lorsque l'assimilation est très faible que la méthode employée est sujette à caution, parce qu'il n'y a pas moyen de préparer des liqueurs nutritives parfaitement exemptes d'azote, attendu que les sucres aussi bien que l'eau et les sels renferment de légères doses d'azote suffisantes pour provoquer une multiplication; les cellules ensemencées, elles aussi, apportent dans leur intérieur un peu de matière nutritive. Aucune des autres méthodes préconisées jusqu'ici ne s'est montrée sûre.

On n'a pas encore découvert de Saccharomycète capable de s'assimiler l'azote libre de l'air.

Les résultats de mes recherches sur l'assimilation se trouvent résumés dans le tableau ci-après, où + signifie que le sucre concerné est assimilé, ÷, qu'il ne l'est pas, et ?, que c'est douteux.

Source d'azote	Eau de levure				Solution d'asparagine				Solution de peptone			
Sucre	D	L	M	S	D	L	M	S	D	L	M	S
Sacch. cerevisiæ.....	+	÷	+	+	+	÷	+	+	+	?	+	+
— Carlsbergensis	+	÷	+	+	+	÷	+	+	+	÷	+	+
— ellipsoideus.....	+	÷	+	+	+	÷	+	+				
— Pastorianus	+	÷	+	+	+	÷	+	+				
— turbidans.....	+	÷	+	+								
— Marxianus.....	+	÷	+	+	+	÷	+	+	+	+	+	+
— fragilis.....	+	+	÷	+	÷	÷	÷	÷				
Zygos. Priorianus.....	+	?	+	+	+	÷	+	+				
Saccharomycod. Ludwigii .	+	÷	?	+	+	÷	?	+	+	÷	?	+
Debar. globosus	+	÷	?	+	+	÷	?	+				
Schwann. occidentalis	+	?	+	+	+	?	+	+				
Schizos. octosporus	+	÷	+	?	+	÷	+	?				

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Laboratoire de Carlsberg, Décembre 1917.

(Remis à la Rédaction en mars 1919).

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JOHS. SCHMIDT:

RACIAL INVESTIGATIONS.

IV. THE GENETIC BEHAVIOUR OF A SECONDARY SEXUAL CHARACTER.

BY

JOHS. SCHMIDT.

I. Introduction.

In the animal kingdom, a distinction is made between primary and secondary sexual characters. By the former are understood such structural features as are directly associated with the sexual organs, and differ, therefore, in male and female of the same species. The two sexes of one species can, however, also, differ in other qualities than those directly relating to the structure of the sexual organs. Such qualities are termed secondary sexual characters, and may be of widely differing nature. Everyone knows some instances of secondary sexual characters: the plumage of the cock, and the antlers of the stag are features in which the male differs from the female of the same species.

With regard to the genetic behaviour of the secondary sexual characters, this will, as far as it is known, follow the Mendelian laws of segregation, albeit at times with certain limitations, which may apparently render the question somewhat complicated. For this very reason, it may be of interest to consider an example which I have had an occasion to study, and in which the character in question seems to follow extremely clear and simple rules in genetic respects.

Secondary sexual characters are also found among the fishes, though they are not as a rule very pronounced among our northern species. As an example from our fauna may be mentioned the dragonet (*Callionymus maculatus*), where the male is distinguished by prolongation of the fin rays and specially brilliant colouring. Among the fishes of warmer regions, we find many conspicuous cases of secondary sexual character, as for instance among several

of the tropical or subtropical small fish which have become common of late years as pets in the ordinary indoor aquarium. One of these is the little cyprinodont *Lebistes reticulatus*, (Peters) Regan, which I have on previous occasions made use of for experimental investigations[1]).

Lebistes reticulatus, which has been called »the millions fish«, is a native of the West Indies and northern South America. It eagerly devours mosquito larvæ, and has for that reason been introduced into several warmer countries, in the hope of checking malaria (Regan, 1913, p. 978).

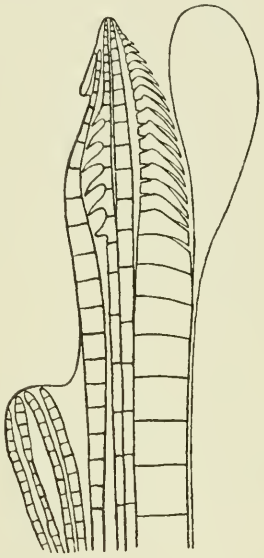
II. Remarks on the Propagation of the Species.

Like so many other cyprinodonts, *Lebistes reticulatus* is viviparous. Copulation takes place, the eggs being thus fertilised within the body of the female. The biological features of several closely related species are well known, some of these species being easily kept in small aquaria. A detailed account is given by Philippi (1908) and Langer (1913) where literature references will be found.

The copulating organ is provided by the anal fin, the third, fourth and fifth rays of which are prolonged and transformed so as to make a channel through which the sperma can be passed. This organ, the gonopodium, as it is called, is freely mobile and can be directed forward. The three prolonged rays of which it consists have, as shown in the accompanying figure, numerous hooks towards the point, which have been presumed to perform some function during the act of copulation. In the female, the anal fin is normal, and the same applies to the other fins. The sperma is collected in sticky, immobile balls, hollow, and with walls consisting of spermatozoa held together by a mass resembling putty. Philippi has given them the name of "*spermatozeugmen*". The male, in pursuit of the female, actually discharges these balls like shot, and, being glutinous, they easily attach themselves to the genital papilla of the female. In a physiological sodium chloride solution, the *spermatozeugmen* remain unchanged for several days, but under the influence of ovarial or oviduct fluid, the cementing

[1]) Racial Investigations Nos. II and III (Comptes-rendus du Laboratoire Carlsberg, **14**, No. 1, 1917 and **14**, No. 5, 1919).

mass dissolves in a few minutes, so that the spermatozoa swarm about (Philippi 1908). Owing to this peculiar arrangement of the spermatozoa in balls, enormous quantities can be introduced, during copulation, into the genital ducts of the female. As the inner surface of the oviduct is furnished with numerous deep longitudinal folds, many of the spermatozoa are retained here, while a number penetrate into the ovaries (Philippi 1908). There is thus a possibility for storage of fertilising spermatozoa long after the time of copulation.



Point of gonopodium in *Lebistes reticulatus*. In the middle, the 3 prolonged, hooked rays of the anal fin; top right, a fold of skin resembling a præputium; left lower corner, anal fin rays not transformed. (After C. Tate Regan, 1913).

In the present species we have found that the period of incubation under normal conditions (abt. 25° C.) is about four weeks. The number of young produced at each birth will depend, inter alia, upon the age of the mother, but may be quite considerable, e. g. 50, or we have even observed 70. A phenomenon well-known to fanciers who keep cyprinodonts in aquaria is that a female whose male has been removed can give birth to several broods of young without further copulation being necessary. This also applies in a high degree to *Lebistes reticulatus*. A female, kept isolated from its arrival at this Laboratory, thus gave birth, in the course of about half a year, to no less than seven broods of young at regular intervals. The ova in question must thus have been fertilised by stored spermatozoa, which had remained living in the female for up to half a year, fertilising the ova as soon as a new batch matured. As will be mentioned later (p. 7—8) we have, however, encountered the peculiar fact that fresh sperma from another male, subsequently introduced, can fertilise the later ripened ova to the exclusion of the older sperma from the first male.

The observer watching for the first time a male and female of *Lebistes reticulatus* together in an aquarium, would hardly believe they were two fish of the same species, so greatly do they differ in size and general appearance. The female can attain a length

of nearly 5 cm, whereas the male is much smaller, hardly $2^1/_2$ cm. The two specimens shown uppermost in Pl. I give an idea as to the relative sizes of the sexes. It is not only in point of size, however, that the male and female are unlike. The female is of a uniform dirty yellow or olive green colour all over the body (see Pl. I, second figure in top row) whereas the male is often resplendent in the most gorgeous hues. It is very difficult to reproduce these colours in a plate, or to describe them closely in words, among other things, because they exhibit considerable variation, and their strength depends, for instance, on the well-being of the specimen at the time. I must also note with regret that the reproduction of the figures has not been altogether satisfactory; there is hardly any doubt that the result should have been considerably better, from the handsome originals painted by Frk. B. Rohweder.

III. Crossing experiments.

In 1916, we commenced work at the Carlsberg Laboratory with *Lebistes reticulatus*, though the object then in view was only to study the inheritance of certain quantitative characters, such as the number of fin rays and vertebræ. To begin with, we had only a single form, purchased from a dealer in Copenhagen. The males of this form were approximately of the appearance shown in the uppermost group of figures in Pl. V. As mentioned above, the colours are difficult to describe. We can, as a rule, however, distinguish between three distinct categories of colouring on the sides of the body: 1) small round black spots, often to the number of three on either side of the body, 2) a ground colour glistening with a green, blue or violet metallic lustre (this lustre is only apparent in reflected, not in transmitted light), 3) various spots of colour, small or large, varying from yellowish-red or reddish-yellow to red.

For the first year, we had only this one form, several generations of which were bred in the laboratory with a view to studying the quantitative characters above referred to. During this time, the genetic behaviour of the colouring in the male did not appear to present any noteworthy features.

Then one day, on visiting an exhibition of aquarium fish, I was shown, as a "novelty", a specially brilliantly coloured form

of *Lebistes reticulatus*, of which I succeeded in procuring a male specimen for the Carlsberg Laboratory. This specimen, which was given number 75 in our experiments, is shown in the uppermost row in Pl. I. As to the origin of the form in question, nothing definite could be ascertained; in all probability it had formed part of a consignment of aquarium fish from Germany. Being anxious to find out something about the origin of our two forms of *Lebistes reticulatus*, I sent specimens of both to Mr. C. Tate Regan, of the British Museum in London, who is the best authority on fishes of this group. Mr. Tate Regan was kind enough to compare my specimens with specimens in the British Museum collection, and was then able to inform me that he could identify both forms with specimens from the island of Trinidad in the West Indies.

On comparing No. 75 (Pl. I) (Form B) with our "old" form (A) Pl. V, upper group) it will be noticed that the two differ considerably. In particular it should be noticed that No. 75 has on the dorsal fin a large black pigment spot most often set in yellowish-white, this spot being lacking in the old form. Furthermore, the red colour is a bright carmine, in contrast to the duller, more yellowish-red colour in the old form, the red colouring also being somewhat differently placed. In the following, I shall deal only with the spot on the dorsal fin.

This male was paired on 22 July 1916 with a female (No. 74) belonging to the form without black spot on dorsal fin (Form A). This female gave birth to 78 young up to its death on $^{11}/_{3}$ 1917, about half the offspring being males. These 36 F 1 males are shown in Pl. I together with their parents, the later at the top of the plate. It will be noticed that the F 1 specimens belong entirely to the father's type, i. e. Form B. All have the black spot on the dorsal fin, and as strongly marked as in the father. As to the female F 1 individuals, there is nothing to remark, since we are here dealing with a feature found only in the males. As a matter of fact, the females of *Lebistes reticulatus* are in the main alike in appearance, whichever form they represent.

The spot on the dorsal fin thus proved dominant over absence of spot, though nothing could be said as to why this should be so. In order to pursue the matter further, therefore, various crossing experiments were carried out.

To begin with, the F 1 specimens were paired one with another, then the F 2 specimens one with another and so on. In

the course of years we have in this manner arrived by in-breeding at F 5, and might have attained to the production of even more generations, had not lack of space and pressure of other work prevented our conducting these investigations on a still larger scale. The species, it should be noted, may attain maturity already at an age of three months.

In Pl. II will be seen a small sample, taken at hazard, from our F 2 specimens, but the figures might equally well represent F 3, F 4 and F 5. It is at once noted that we have here only the form B represented by male No. 75, specimens without the dorsal fin spot (Form A) being altogether lacking. None of the 998 registered specimens of F 2—F 5 showed any segregation of the unspotted form (A).

We have also made back-crosses in both directions, as shown by figures on Pl. III and IV.

The uppermost figure Pl. III is an F 1 specimen (No. 91) which was paired with a female of the unspotted form (No. 132), while the remaining 13 figures show the male offspring of the first brood. As will be seen, there is here again no appearance of the unspotted form, but only the type B represented by male No. 75. The same thing was found with the remaining offspring of back-crosses between F 1 males (Form B) and females of Form A, amounting to 70 in all. Similarly, 133 male specimens, offspring of F 2 males (Form B) paired with females of Form A were all found to belong to Form B, with the spot on the dorsal fin.

The result of the second experiment in back-crossing is seen in Pl. IV. The female used was an F 1 individual of 74 × 75 (No. 109), the male a specimen without spot on dorsal fin (No. 110). The male is shown at the top of the plate, the other figures representing the male offspring of the first brood. Here again no segregation has taken place; all the specimens lack the dorsal fin spot, and the same applies to all the remaining male specimens produced by back-crosses of F 1 females with males of the unspotted form, 265 specimens in all. Further, offspring specimens of 109 × 110 were paired one with another, with the result that all the male offspring, totalling 67, lacked the spot in the dorsal fin.

The main result of all our crossing experiments then was, that no Mendelian segregation could be shown, either in F 2 or later generations by in-breeding or back-crosses in both directions.

The effect in any case was, that males lacking spot on dorsal fin had only unspotted offspring, while the offspring of males with the dorsal fin spot were always similarly spotted.

IV. Other Experiments.

Following on the crossing experiments just described, I will mention some others which supplement them in a remarkable degree.

After it had been found that the male offspring only inherited qualities from the father, as far as the dorsal fin spot was concerned, we paired the same female with two males at once, one with, the other without spot on dorsal fin, in order to see whether both forms would be represented in the offspring. This actually happened, as will be seen from the lower group in Pl. V. The two uppermost figures are two males, paired simultaneously with the same female. The figures below them represent the offspring, and show that both forms are present, the spotted and unspotted. There is, perhaps nothing remarkable in this. If sperma from two males be introduced at about the same time, there will always be a chance that a portion of ripening ova may become fertilised by spermatozoa from both males.

The further results of these experiments, however, are more remarkable. In section II we noted that a female whose mate had been removed after copulation might give birth to as many as 7 broods of young at intervals of about a month, the explanation being, that the sperma is stored in the genital ducts of the female, and can thus fertilise the periodically ripening ova. If, for instance, a female be paired with a male of Form B (with dorsal fin spot) and the male thereafter removed, then naturally, from what we have seen above, the female will produce spotted offspring, and continue to do so after the removal of the male. But what happens if this paired female, deprived of her mate, be later paired again with another male, of the unspotted form A?

By way of answering this question, I have carried out several corresponding experiments. I will here give the details of one of them.

A female (No. 92) was paired with a male (No. 91) belonging to form B (with spot on dorsal fin). The resulting data were as follows:

Female 92 paired with Male 91 (Form B)

$^{5}/_{4}$ 1917 brood 1	
$^{26}/_{4}$ 1917 brood 2	
$^{1}/_{6}$ 1917 Male 91. removed	All ♂ offspring (34)
$^{17}/_{6}$ 1917 brood 3	were of Form B,
$^{14}/_{7}$ 1917 brood 4	with spot on dorsal fin.
$^{13}/_{8}$ 1917 brood 5	
$^{16}/_{9}$ 1917 brood 6	

$^{17}/_{9}$ 1917 new male (No. 138) (Form A, without dorsal fin spot), introduced.

$^{16}/_{10}$ 1917 brood 7	
$^{15}/_{11}$ 1917 brood 8	All ♂ offspring (67)
$^{15}/_{1}$ 1918 brood 9	were of Form A
$^{18}/_{2}$ 1918 brood 10	without spot on dorsal fin.
$^{21}/_{3}$ 1918 brood 11	

The above data seem to me to point to a distinct conclusion. The sperma introduced from male No. 138 must at once have gained supremacy and fertilised the next batch of ova, ripened since the birth of brood 6. Brood 6 contained Form B (with dorsal fin spot) only, whereas brood 7, born a month later, consisted only of Form A (without spot).

In another experiment, a single female (No. 145) was paired successively with several (5) males, chosen alternately from Form B and forms without the dorsal fin spot. The female was then seen to bring forth with the greatest regularity alternate broods of exclusively spotted and exclusively unspotted offspring. It should be added, that in these experiments, we always introduced a new male immediately after the female had brought forth her young, and in some cases immediately after the old male had been removed.

In explanation of the peculiar state of things here described I have had recourse to the assumption that the fresh spermatozoa are more agile than the older stock which had been stored for varying lengths of time in the genital ducts of the female, and are thus unable to compete with the former, though they would, if left to themselves, have been capable themselves of fertilising the next batch of ova. The fact that we can, by pairing a female simultaneously with two males of different form, produce, in one and the same brood, offspring belonging to both forms, seems

to support this explanation of the selection which takes place among the spermatozoa in the genital ducts[1]).

V. Discussion of the Results of the Crossing Experiments.

As mentioned in the foregoing, the main result of our crossing experiments was that males without dorsal fin spot only produced unspotted offspring, whereas on the other hand, the offspring of males with spotted dorsal fin were all similarly spotted.

We find here, then, a doubly unusual state of things, inasmuch as 1) no Mendelian segregation could be shown, and 2) the offspring inherited exclusively from the one parent.

As regards the latter point, there are, it is true, instances known of offspring showing inheritance from one parent only. I have here in mind the heredity of variegation of leaves in certain plants, as in the case of the hop species, *Humulus japonicus*. recently investigated at this Laboratory by Ö. Winge (1919). Variegation of leaves was, however, transmitted only through the mother, and that, moreover, in a manner which renders it extremely probable that the transmission of this quality to the offspring takes place through the protoplasm of the egg-cell, the nucleus playing no part in the process, in contrast to what appears to be generally the case. (This explanation was first advanced by Correns, in regard to *Mirabilis Jalapa*).

That the hereditary transmission of the spot on the dorsal fin of *Lebistes* should be effected through the protoplasm as in the case of variegated plants must, however, be regarded as out of the question, if only from the fact that it has been found to be transmissible only from the father. There is thus no reason to doubt that the heredity of the quality in question is as usual

[1]) On going through the interesting works of Correns (1917, 1918) on experimental dislocation of the relation between number of males and number of females in a dioecious plant *(Melandrium)* I find here what is apparently a parallel to the selection of spermatozoa in *Lebistes reticulatus*. Correns states, that there is a difference in the rapidity with which the pollen tubes of the various pollen grains grow down through the style tissue on their way to the ova. The pollen grains which are determiners of femaleness, i. e. those whose fertilisation of an ovum produces a female seed, have, according to Correns, the advantage as compared with determiners of maleness, in that their pollen tubes grow more rapidly. The determiners of femaleness therefore have a start in the competition for the ova, which renders possible — as with *Lebistes* — a selection among the male gametes present.

bound to the nucleus, and the question then is, how it could be that no Mendelian segregation was discernible.

I should here observe that it is intended to pursue these experiments further, as there are various other points which I wish to have investigated more closely. How the future experiments turn out must be left to the future to show, but it will be natural already to seek some explanation of the results arrived at up to now, which are, as shown in section III, based on no inconsiderable quantity of material.

An idea which immediately suggests itself is that the quality concerned might be dependent upon more than one, possibly several, factors, and that the cause of non-segregation might thus be sought in the fact that the material was insufficient to exhibit such segregation. Against this, however, we have the fact that F 1 is not intermediate between the two forms, but apparently identical with the father's type. If, nevertheless, we adhere to the view that the absence of segregation is due to the fact that numerous factors were at work, it must be supposed that even the presence of a single or quite a few factors would suffice to give full dominance to the spot. This would of course not be impossible beforehand, but in that case we should have the right to expect that our back-cross No. 2 (Pl. IV) where F 1 females of 74×75 were paired with unspotted males, would have thrown off individuals with spotted dorsal. This, however, was not the case; all the 265 male specimens investigated were typical representatives of the unspotted form.

As we have thus been unable up to now to discover any Mendelian segregation, we must look for some explanation covering the facts as shown. The simplest may be found in the assumption that the quality here concerned is dependent upon a factor localised in an extra sex chromosome in the male. Let us imagine, for instance, that the female has the formula XX and the male XY, and that only Y contains the factor in question. The female will then produce only the X gametes, all lacking the factor, and the same will apply to half the gametes of the male, viz. those indicated by X. The other half of the gametes from the male, however, (Y), to wit, those whose fertilisation of egg-cells produced male offspring, all possess the factor in question. The result of this would be, that the quality could only be transmitted through the father.

The present results of these experiments are then in entire agreement with this explanation, and wherever such conditions as here assumed prevail, the transmission of a quality must take place exactly in the manner shown by our experiments with *Lebistes*[1]). As far as I am aware, no factors have hitherto been discovered localised in a male Y chromosome, but there is nothing to indicate, in principle, that this could not be the case.

In conclusion, I would point out that the one-sided hereditary transmission found in *Lebistes reticulatus* must in a certain degree alter, or at any rate extend, our conception of the "race" in fishes. There can be no question, of course, but that the two types, A and B, are characteristically distinct. We can therefore hardly hesitate to separate them as independent forms or races, more especially after they have been found to be hereditarily permanent. This brings us then to the apparently remarkable state of things, that racial difference here exists only in the one sex, the male, no such idea being tenable in the case of the opposite sex.

[1]) In order to throw light on the question, if possible, from other points of view, Dr. O. Winge has commenced an investigation of the cytological conditions. The question whether the above interpretation is correct may possibly be answered by a cytological study.

Carlsberg Laboratory, 12. February 1920.

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EXPLANATION OF PLATES.

Pl. I. Uppermost ♂ 75 and ♀ 74; below, their male offspring (F 1), all of the father's type.

Pl. II. 18 F 2 males (of 74 × 75), all of the 75 type ("Form B").

Pl. III. Back-cross No. 1.
Uppermost, an F 1 male (No. 91) paired with a female (No. 132) of "Form A"; below, their male offspring of the first brood, all of the 75 type ("Form B").

Pl. IV. Back-cross No. 2.
Uppermost, a male without spot on dorsal, paired with an F 1 female (of 74 × 75); below, their male offspring of the first brood, all of the father's type (without dorsal fin spot).

Pl. V. Upper group:
Uppermost, a male of "Form A" (without dorsal fin spot) paired with a female of same form. The male is distinguished by marked faintness of the red colouring. In the offspring, this colouring was considerably stronger. (This experiment is not referred to in the text).

Pl. V. Lower group:
Uppermost, two males (No. 146 and 147), one of "Form A," the other of "Form B," paired simultaneously with the same female (No. 145). Below, male specimens of the offspring, showing that both forms are there represented.

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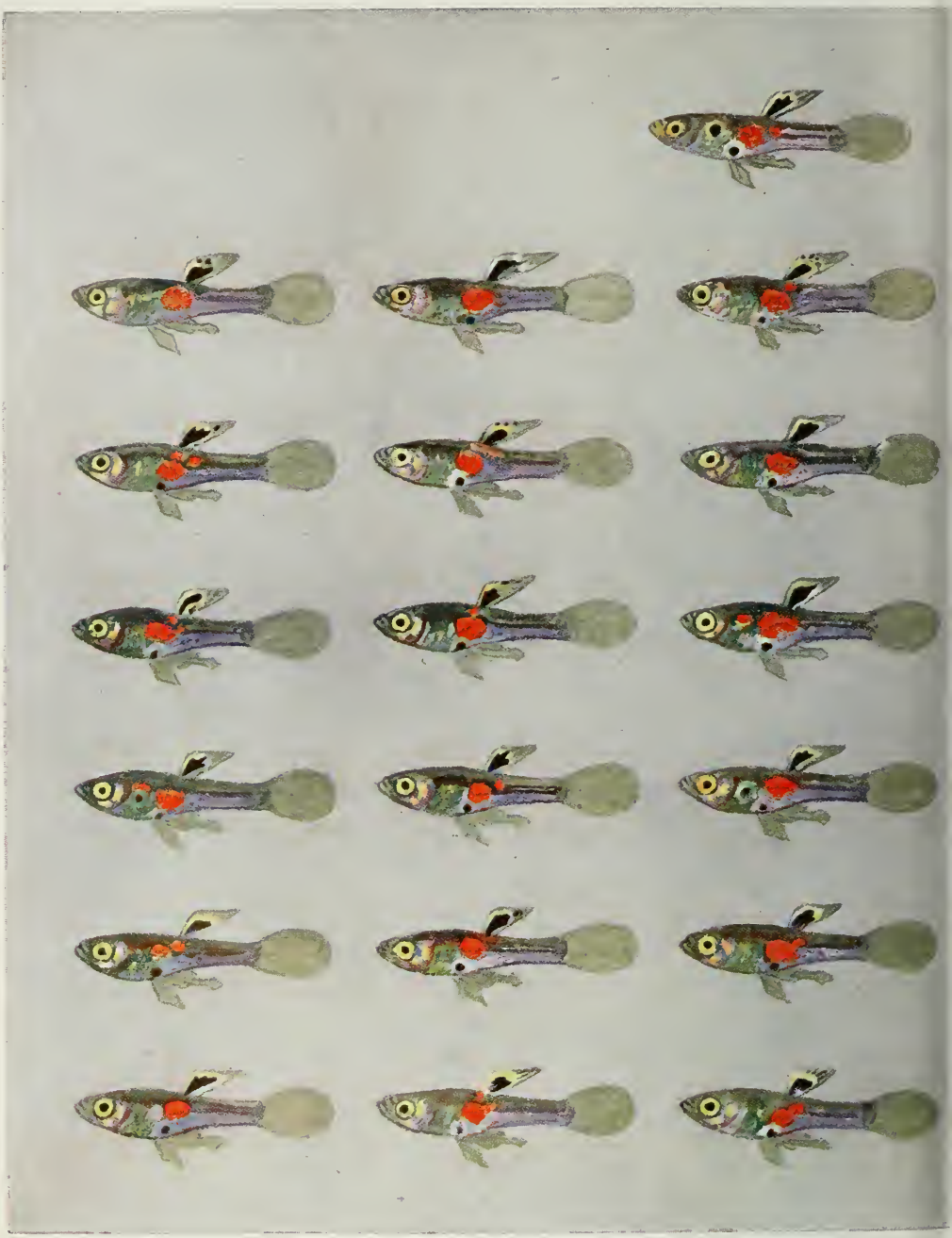

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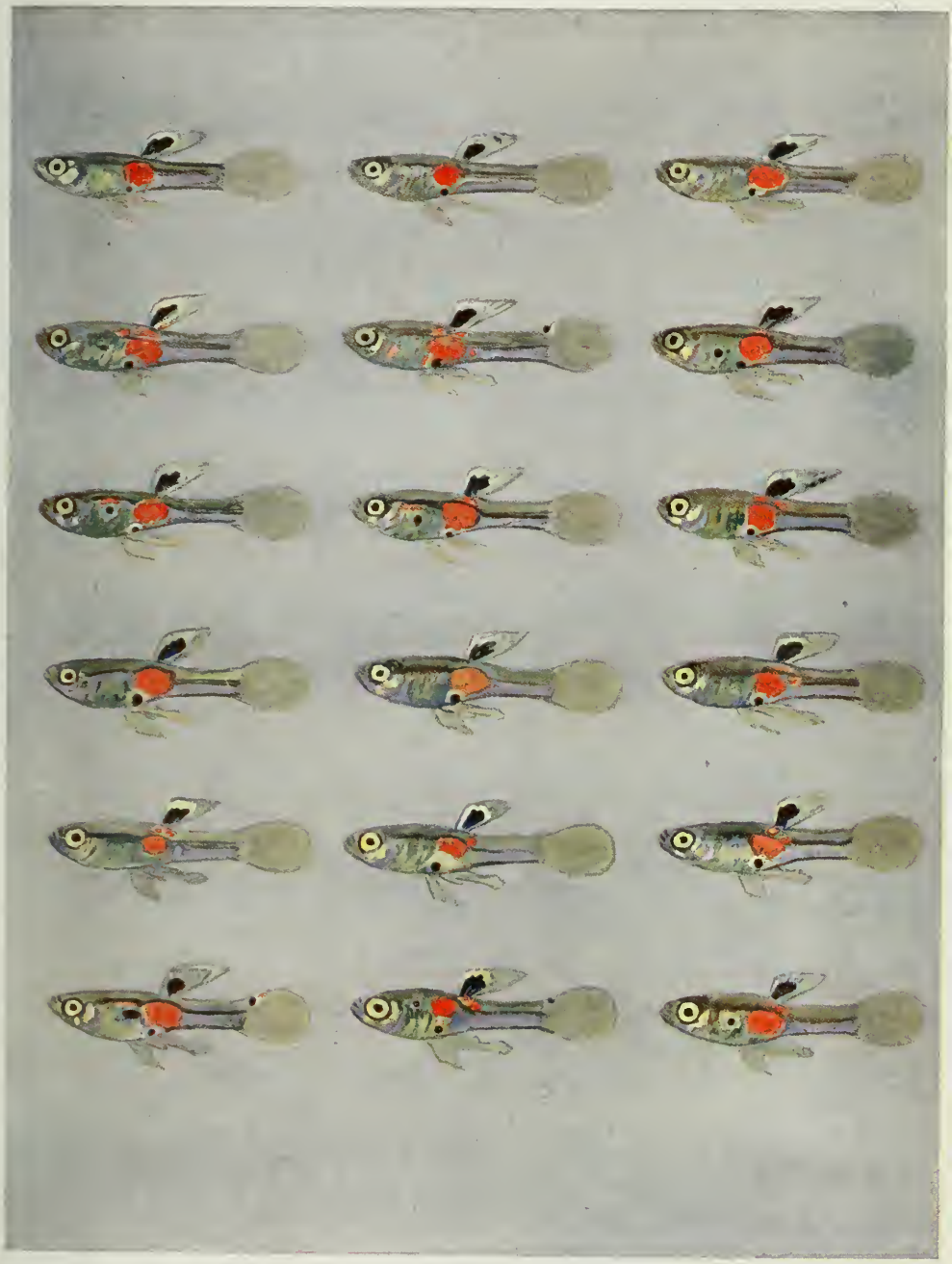

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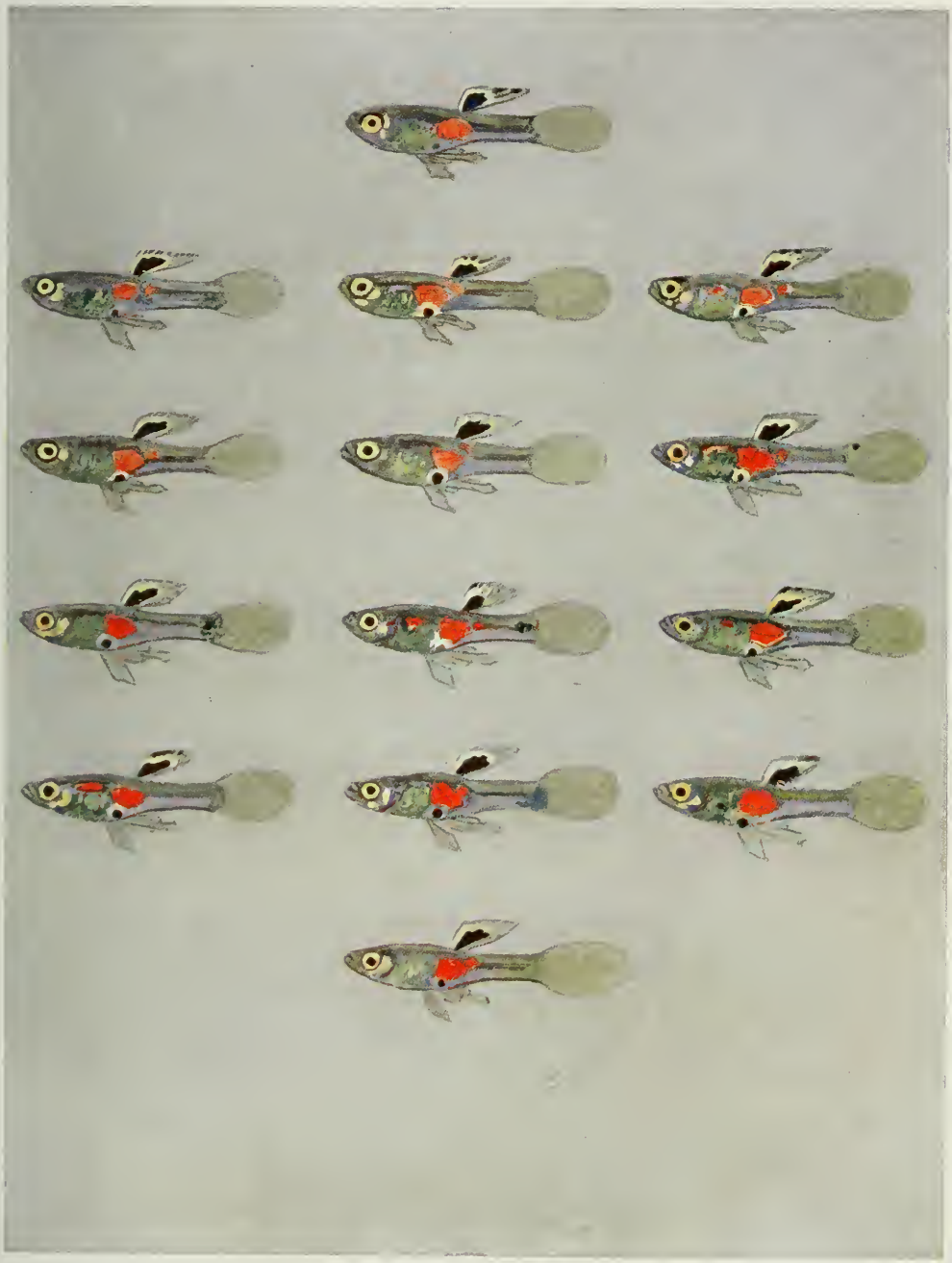

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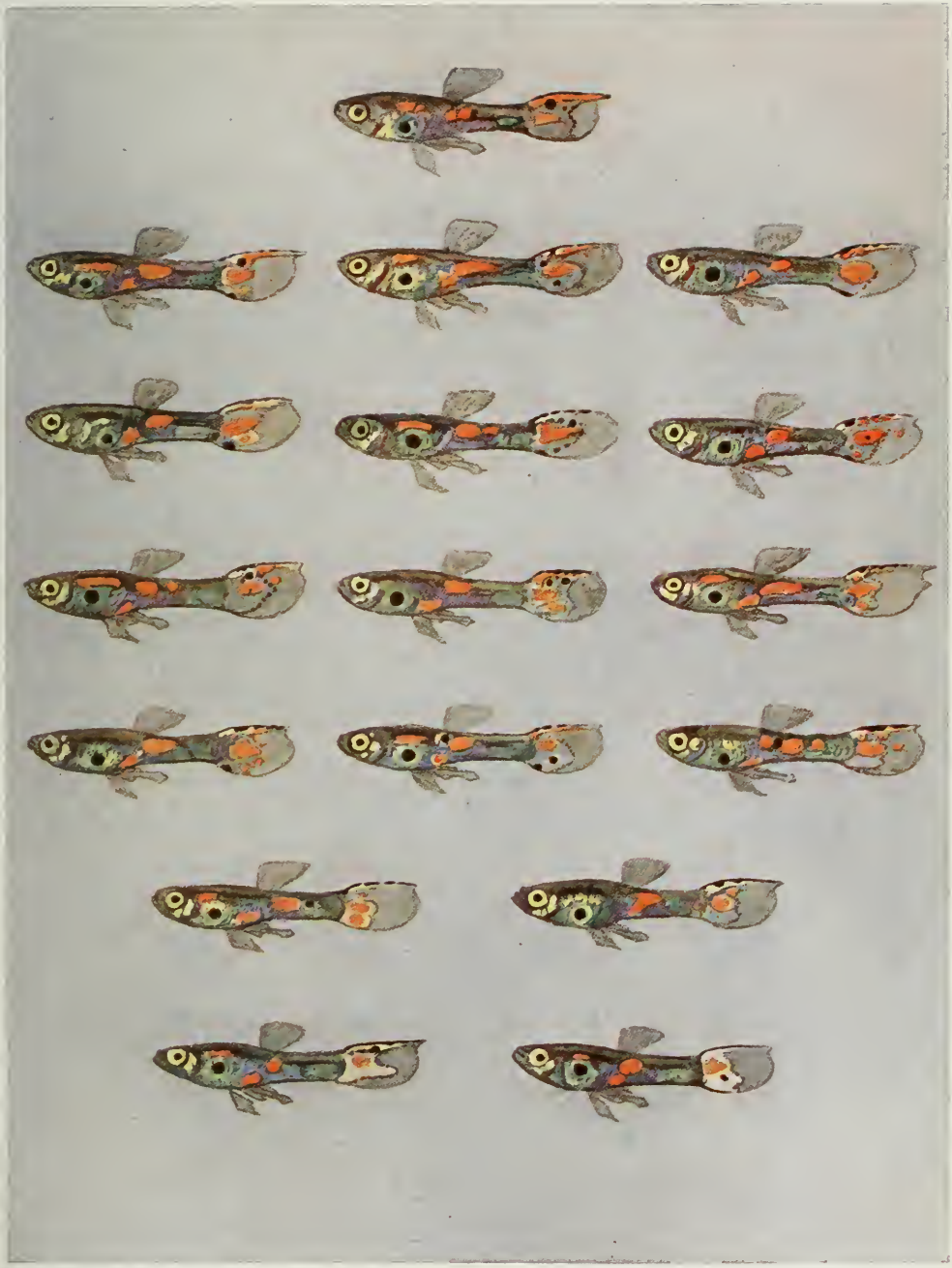

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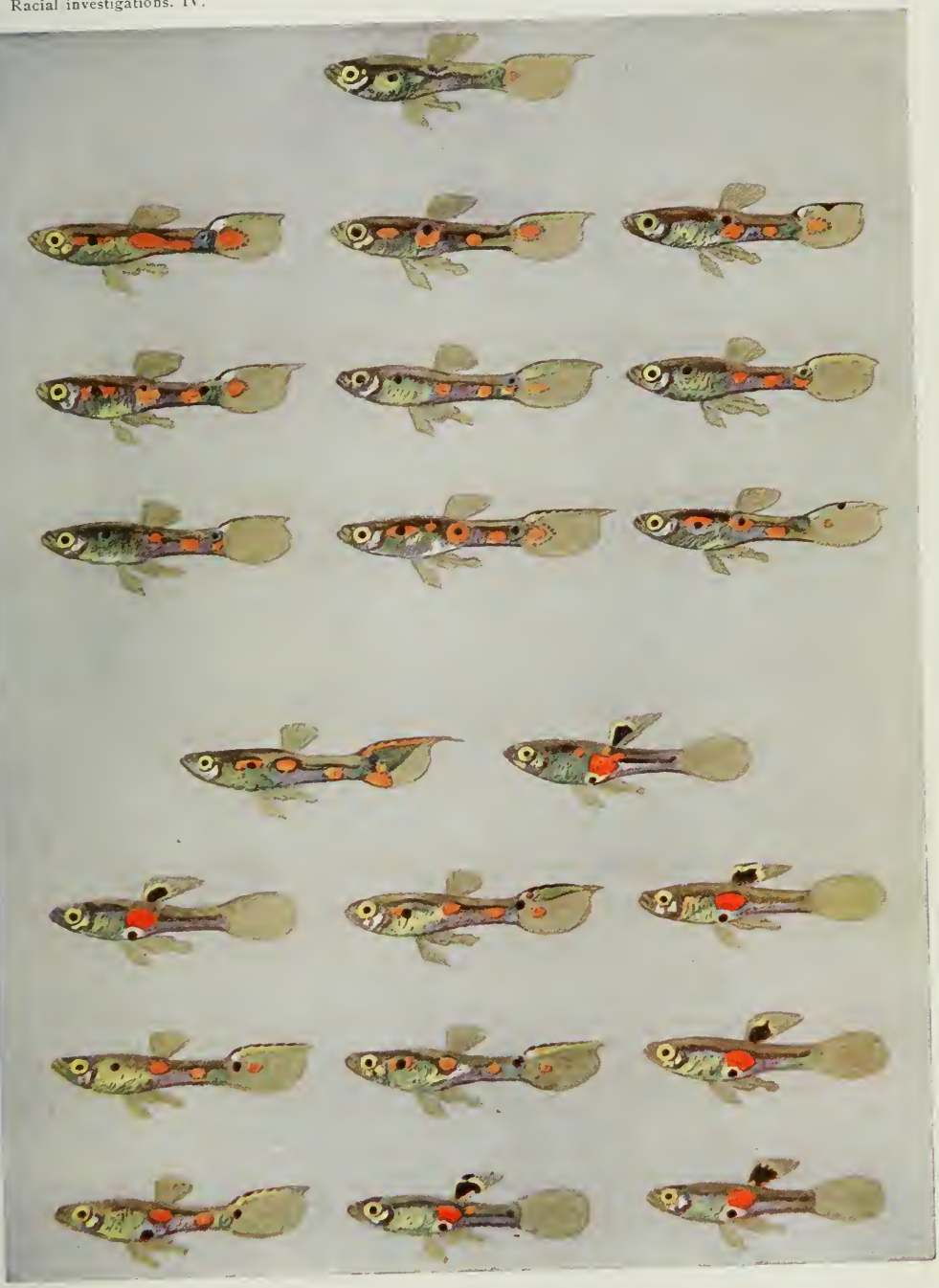

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JOHS. SCHMIDT:

RACIAL INVESTIGATIONS.

V. EXPERIMENTAL INVESTIGATIONS WITH *ZOARCES VIVIPARUS* L.

BY

JOHS. SCHMIDT.

I. Introduction.

In a previous paper[1]) the principal results of my statistical investigations with *Zoarces viviparus* were summarized. I there expressed the following view, with regard to the nature of "races" in *Zoarces* and other fishes as characterized by our population analyses: "A fish "race" is largely a statistical conception. It implies a mixing of different genotypes, and the average values characterising the "race" are primarily dependent upon the quantitative proportion between these; only secondarily on the environment." It was further pointed out that we could not expect to arrive at any final decision before the results of those experimental investigations which were carried out along with the statistical investigations with the same species since 1914, were available. This is the case now, at least with regard to some of the experiments which are so advanced as to be suitable for publication. I propose to give here a summary of some of the most important results.

Two *Zoarces* populations are to be dealt with here, both from Danish waters, viz the Ise Fjord (Station 31) and Roskilde Fjord, north coast of Sealand (Station 34), the latter being a branch of the former (see Fig. 1, p. 8). Both populations were mentioned in the earlier papers quoted above and graphs representing the number of vertebrae were given[1]).

[1]) See Johs. Schmidt: *Zoarces viviparus* L. and local races of same (Comptes-rendus du Laboratoire Carlsberg, **13**, p. 389, 1917), and »Statistical investigations with *Zoarces viviparus* L.« (Journal of Genetics, VII, No. 2, 1918).

In addition to the ordinary population-analyses individual offspring analyses have been carried out each autumn since 1914, the offspring of each gravid female being separately investigated. From each female, 10 embryos (a "decade") were taken at random and their number of vertebrae etc. determined[1]).

For the sake of brevity I shall restrict myself to mentioning here a single character, viz. the number of vertebrae. This character has proved to be of great importance in distinguishing local races in *Zoarces*, where the number has been found to vary from 99 to 126. A great material of many thousands of specimens was analysed and statistically treated. The countings were made by myself (1914—1917) and by Mr. Vilh. Ege, M. Sc. (1917—1919).

II. Comparison of the different annual classes within a population.

For this purpose the decade investigations, consisting in an analysis of ten embryos taken a random from each gravid female are excellently suited. By examining the embryos instead of the adult specimens all doubt is of course excluded that the individuals belong to the same annual class.

Table I p. 11 shows the results of offspring analyses (decade investigations) within the population from Station 31 during the years 1914—1919. Altogether 871 decades or 8710 individuals rather evenly distributed between the six years have been examined.

It is seen from this table that the population-averages for the different annual classes agree very well. By means of the present great material of observations it has thus not been possible to point out any difference between the various annual classes with regard to the average number of vertebrae. In other characters such a difference can, however, be pointed out, e. g. for the number of pectoral rays[1])

In Table II p. 11 the same material is arranged in another way. If it is represented by a curve it will be seen that this is very regularly shaped, as might be expected when the material is so large.

Table III p. 12 also deals with the same material. It shows the average values of ten decades, each figure representing a

[1]) Johs. Schmidt: Constancy investigations continued, in Comptes-rendus du Laboratoire Carlsbeg, **14**, No. 1, p. 2—11, 1917.

hundred individuals. It will be seen that the highest value is 114.17, the lowest 112.28 which are respectively 0.97 above and 0.92 under the average of the population which is 113.1963. The values of Table III are especially interesting when compared with the corresponding figures for individuals coming from the same population, but developed under different outer conditions (see section V, p. 7).

III. Comparison of mothers and their offspring. — Significance of internal factors.

This part of the investigations is directed to the question whether the character examined, viz. the number of vertebrae, is hereditary, i. e. due to internal factors. As the father in each single case is unknown it is only possible to compare the offspring with the mother. As the material is so large we can however assume that the paternal number of vertebrae is on an average equal to the average of the population, i. e. abt. 113.2. Provided the number of vertebrae is a hereditary character our investigation should show that mothers with a great number have offspring with a great number. To test this we arrange the material given in Tables I—III so that for each number of vertebrae occurring in mothers we indicate the frequency of it and the average number of vertebrae in the offspring derived from these mothers.

From Table IV p. 12, representing a very considerable material namely no less than 857 mothers and 8570 individuals of their offspring, it clearly appears that the average number of vertebrae in the offspring depends upon the number of vertebrae of the mothers since the values of the offspring continuously increase as the maternal numbers of vertebrae increase from 107 to 119. A conformity like that could not appear if the number of vertebrae in an individual were alone determined by the environmental conditions during the development.

The investigations with *Zoarces* thus show that there are differences of hereditary nature between the various individuals, and they confirm the results of R. C. Punnett, arrived at by investigation of the viviparous shark *Spinax niger*[1]), as well as

[1]) R. C. Punnett: Merism and Sex in *Spinax niger* (»Biometrika«, vol. III Part IV, 1904).

those obtained from my experiments with *Lebistes* and the common trout[1]).

The Table thus shows that the number of vertebrae in *Zoarces* is a hereditary character. We must, however, not expect that the mothers with for instance 119 vertebrae have offspring with on an average 119 vertebrae. As will be seen from the table that is not the case, the average being only 116.3. In the first place the paternal number is not 119, but abt. 113.2 (the average of the population), a circumstance that causes the offspring to "regress" towards the average value of the population. Further regression is due to the fact that the maternal numbers of vertebrae, in casu 119, are personal values, while their generative values in all probability will deviate less from the average of the population than do the personal values[2]). Provided the material be sufficiently great, the following relation must presumaly hold:

$^1/_2$ (Average ♀♀ 119 + 113.2) = Average Offspring ♀♀ 119,

where "Average ♀♀ 119" is the average of the generative values of mothers with 119 vertebrae and "Average Offspring ♀♀ 119" is the average of the offspring originating from those mothers.

I am not going to discuss the results of Table IV closer here. As the present large material, however, seems to deserve a more thorough statistical treatment than I am prepared to give it I have asked the statistician Miss Kirstine Smith, D. Sc. to deal with it and I hope the results will before long be ready for publication in the "Comptes-Rendus du Laboratoire Carlsberg".

IV. Comparison of two different populations developed under the same environmental conditions. — Significance of internal factors, continued.

The two populations in question differ essentially in the average number of vertebrae, which for the first of them, the population dealt with above, from the mouth of the fjord (Station 31) is abt. 113.2, and for the other population, from the innermost

[1]) Johs. Schmidt: Experiments with *Lebistes reticulatus* (Peters) Regan (Comptes-rendus du Laboratoire Carlsberg, **14**, Nr. 5, 1919) and Diallel crossings with trout (*Salmo trutta* L.) (Journal of Genetics, Vol. IX, No. 1, 1919).

[2]) About the concepts "personal" and "generative" value see Johs. Schmidt: "La valeur de l'individu à titre de générateur, appréciée suivant la méthode du Croisement diallèle (Comptes-rendus du Laboratoire Carlsberg, **14**, No. 6, 1919).

part of the fjord (Station 34, see figure 1, p. 8) abt. 108.0. The experiment was carried out in the way that in June of 1916, i. e. at a time of year when the sexual products of *Zoarces* are not yet ripe, abt. 300 specimens of each population were caught. They were removed to an embanked area in the neigbourhood to Station 31 and put into big perforated wooden boxes, placed on the bottom near each other, projecting above the water so that feeding could easily be carried out. The pairing and the development of the embryos thus took place in captivity and under the same external conditions for both population-samples. In December 1916, i. e. at a season when *Zoarces* contains large embryos, the boxes were lifted out of the water and their contents preserved. In the population-sample from Station 31 was found 27 females with embryos, and in that from Station 34 a number of 11.

Under natural conditions the average number or vertebrae in the population at Station 31 is abt. 113.2 (see Table I), while in the population at Station 34 it is abt. 108.0 (see my first papers from 1917 and 1918). The values for the two populations thus differ by abt. 5 vertebrae, a difference which the annual investigations since 1914 have proved constant. In spite of the small distance of abt. 50 kilometers between the two stations, their natural conditions differ widely, e. g. at the first of them the salinity of the water is abt. 21 ‰, and at the other only abt. 12 ‰. It might therefore be conceivable that the cause of the difference in the number of vertebrae would be found in a direct action of the different environmental conditions. If this was the case, it was to be expected that the difference would disappear when the two populations were brought under similar outer conditions. This was done in our transplantation experiment, and it is the question now, whether the difference of 5 vertebrae, appearing under normal conditions, has disappeared in the offspring, all of which have developed in our experiment under the same external conditions.

The result of the experiment is given in Table V p. 13. For comparison it may be stated that the average number of vertebrae in offspring developed under natural conditions in 1916 for Station 31 was 113.101 $\pm$ 0.447[1]) (125 decades), and for Station 34 was

[1]) The uncertainty of the average is here and in the following indicated by the fluctuation equal to 5 times the probable error.

107.768 ± 0.468 (116 offspring-samples each consisting of 5 specimens), showing as usual a difference of abt. 5 vertebrae.

It is seen from the table that the offspring of the transplanted population-sample from Station 31 had on a average 114.819 ± 1.059 vertebrae, while the corresponding value for the sample from Station 34 was 108.283 ± 1.363. In other words the difference between the two populations has not disappeared although the development took place under the same circumstances. The result of our transplantation experiment with *Zoarces* thus agrees with the experiment with *Lebistes* previously described and confirms that the difference, proved to exist between the two populations, is of hereditary (genotypical) nature.

V. Comparison of two samples of the same population developed under different environmental conditions. — Significance of external factors.

The transplantation experiments just mentioned were continued in 1917 with material from Station 31. A great number of *Zoarces* individuals were in early summer, and therefore when the sexual organs were still unripe, brought into four large wooden boxes placed in the same embanked area near Station 31 as in 1916.

When the experiment was brought to a conclusion the total number of gravid females was 92. The number of offspring samples examined from transplanted specimens was, including the 27 decades from the 1916 experiment, altogether 119.

The investigation showed that the average number of vertebrae of the 119 decades was 114.535 ± 0.493[1]). The corresponding figure for the non-transplanted samples of the same population was in 1916 113.101 ± 0.447 and in 1917 113.251 ± 0.372. It is therefore evident that the outer conditions which were altered through the transplantation have decidedly raised the average number of vertebrae in the population.

The same thing becomes apparent when the material is arranged in another way. With the same arrangement of the material from the transplantation experiment as was used in Table III,

[1]) For the sake of completeness it is stated that the average for the transplanted mothers was 112.74 ± 0.65, and for the males (111 specimens), found in the boxes at the conclusion of the experiment, 112.95 ± 0.61.

we arrive at the following figures, each of which represents 100 individuals (10 decades):

114.63	114.08
114.55	114 59
114.65	114.65
114.09	114.46
114.09	114.42
113.88	114.42 (average of 9 decades only).

These figures have to be compared with the figures of Table III p. 12 for non-transplanted samples. Such a comparison does not leave any doubt that the number of vertebrae has really been raised by the transplantation: out of the 12 values above 11 are greater than 114, while of the 87 values in Table III only 5 are greater than 114.

It is further shown in Table VI p. 13 that the average number of vertebrae in offspring of mothers with a given number of vertebrae e. g. 116, is greater for transplanted than for non-transplanted specimens.

It is therefore proved to a certainty that the tranplantation from the natural conditions at Station 31 to the embanked area raises the average number of vertebrae in the population. It is not possible to decide for certain what factor or factors are at work to that effect. But it is a fact that the temperature in the boxes, placed in quite shallow water, about $2^1/_2$ meters deep, was higher than at Station 31 and on the whole probably higher than for any natural population of *Zoarces* in Danish waters. On the other hand the salinity was lower in the boxes than a Station 31 viz. abt. 12 ‰ against abt. 21 ‰ at Station 31. In the embanked area, where the boxes were placed, and which is used as an eel-farm, *Zoarces* is only found occasionally and in extremely small numbers, whereas it abounds outside the dam, at Station 31.

VI. The *Zoarces* populations of the Ise Fjord - Roskilde Fjord area.

Beside the yearly analyses of the populations from Stations 31 and 34 we in 1919 carried out analyses of a greater number of populations from the same fjord area. As will be

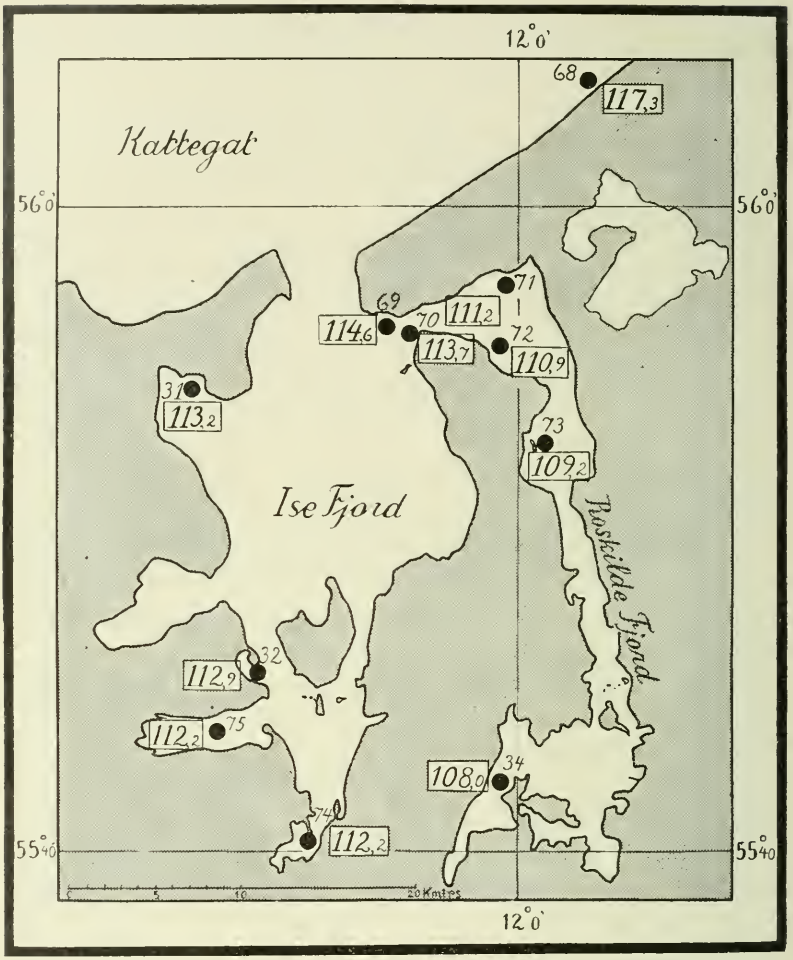

Fig. 1. — Average number of vertebrae in 11 *Zoarces* populations from the Ise Fjord - Roskilde Fjord area, Sealand, Denmark.
The figures at the black spots indicate station numbers, those within the rectangles the average number of vertebrae of the population concerned.

seen from Fig. 1, the Ise Fjord opens into the Kattegat (at the north coast of the isle of Sealand), and Roskilde Fjord is a branch of Ise Fjord. In both fjords *Zoarces* occurs in abundance right from the mouth to the base.

Table VII p. 14 shows the result of our population-analyses which are also represented in Figure 1, p. 8. It will be seen that the populations living outside the two fjords, in the Kattegat, have the average number of vertebrae which is characteristic of the

Fig. 2. — Ise Fjord - Roskilde Fjord area, Sealand, Denmark. Showing the salinity of the water in per mille (‰) at the end of November 1919.

populations of the open sea in the Baltic area, viz. 117—118. In the mouth of the fjord the number is 114—115. From this the value falls quite evenly in the Ise Fjord until 112.2 which is the lowest value for that fjord. In the Roskilde Fjord it falls suddenly to 111.2 (Station 71) and 110.9 (Station 72), reaching near the base of this fjord, at Station 34, the lowest average value hitherto known for any *Zoarces* population, viz. abt. 108.0.

This detailed investigation thus quite confirms the results of

my previous investigations (C. R. Labor. Carlsberg 13, 1917) and shows strikingly in all details how the average number of vertebrae decreases from the coast and the mouth of the fjord towards the base of the fjord. Here *Zoarces* has a short and plump shape, while the populations living farther out consist of individuals slender and elongated in shape (see fig. 16—17, p. 309 in C. R. Labor. Carlsberg, 13, 1917). This quite regular gradation of the average number of vertebrae has now been found in all Danish fjords where investigations have been undertaken and it must therefore be considered a definitely established fact.

The chemical and physical conditions of our fjords are not closely investigated. Among the conditions which are best elucidated the salinity of the water exhibits the most characteristic picture. As will be seen from Fig. 2 this factor shows a gradation which strikingly corresponds to the gradation in the average number of vertebrae. From that to conclude that this character is directly determined by the salinity or by a factor acting parallel to it — which several investigators have been inclined to do — is however not justifiable as I have already explained in my first paper on *Zoarces* from 1917.

VII. Concluding Remarks.

The investigations and experiments with *Zoarces* described above have rendered new evidence of the correctness of the view that the race-characters in fishes are of a hereditary nature i. e. that they are first and foremost determined by internal factors (Sections III and IV). That they are however on the other hand to a certain extent influenced by external factors, is here for the first time experimentally proved for one of our northern species of fish. We have thus seen that the average number of vertebrae which under natural conditions remained very constant in 6 consecutive annual classes of the same population (Section II) was altered, if not strongly, at any rate perceptibly, by a transplantation of the population (Section V).

In view of the investigations hitherto undertaken the gradation of the average number of vertebrae has to be interpreted not as a direct effect of the external conditions (the salinity or another factor acting parallel to it), but as the result of a selection.

Table I. — Station 31, Nakkehage, Ise Fjord, Denmark, 1914—1919. — Decade investigations.

Average number of vertebrae *(a)* in the six annual classes 1914—1919.

(*n* number of decades. *a* average number of vertebrae, σ standard deviation for average value of a decade, P. E. A. Probable error of average, P. F. A. Probable fluctuation of average.)

Year	1914	1915	1916	1917	1918	1919	1914—1919
n	139	177	125	178	154	98	871
a	**113.180**	**113.275**	**113.101**	**113.251**	**113.248**	**113.124**	**113.196**[2])
σ[1])	1.452	1.459	1.480	1.472	1.453	1.265	
P.E.A.[1])	0.083	0.074	0.089	0.074	0.079	0.086	0.032
P.F.A.[1])	0.415	0.370	0.447	0.372	0.395	0.431	0.160

Table II. — Station 31, Nakkehage, Ise Fjord, Denmark, 1914—1919. — Decade investigations.

Number of vertebrae in 857 decades. Same material as in Table I[3]).

Number of vertebrae	Number of specimens
122	1
121	1
120	6
119	22
118	100
117	269
116	647
115	1109
114	1616
113	1785
112	1397
111	893
110	463
109	172
108	65
107	17
106	6
105	1

[1] calculated from exact decade averages.

[2] average of the six averages (1914—1919).

[3] 14 decades — offspring of 14 mothers in which the number of vertebrae could not be determined due to accidental damage — were excluded.

Table III. — Station 31, Nakkehage, Isefjord, Denmark, 1914—1919. — Decade investigations.

Each figure represents 100 individuals (average of 10 decade averages).

114.09	113.34	113.42	112.71	113.85
113.51	113.77	112.41	113.83	113.63
112.65	113.69	113.17	113.55	112.77
112.54	113.04	113.56	113.65	113.03
113.00	113.34	112.59	114.17	112.62
112.82	113.55	113.13	113.96	112.75
112.82	113.36	113.52	112.75	113.25
113.65	114 08	112.70	113.49	112.38
113.12	113.69	113.05	112.44	113.34
113.39	112.28	112.74	113.45	113.52
113.59	112.95	112.98	113.45	112.99
113.12	112.82	112.59	113.24	113.23
112.74	112.45	112.91	113.31	113.47
113.48	112.33	113.05	113.42	113.40
114.07	113.38	113.11	113.56	112.68
113.93	112.94	113.43	113.22	
113.22	113.73	113.86	114.08	
112.70	112.86	112.76	112.93	

Average of populatiou ca. 113.196.

Table IV. — Station 31, Nakkehage, Isefjord, Denmark, 1914—1919. — Decade investigations.

Number of vertebrae in 857 mothers as compared with average number of vertebrae in their offspring (857 decades). — Average of population ca. 113.196.

Maternal number of vertebrae	Frequency of mothers or decades	Average number of vertebrae in offspring
119	3	116.300
118	6	115.617
117	31	114.794
116	65	114.155
115	109	113.851
114	151	113.515
113	185	113.121

Maternal number of vertebrae	Frequency of mothers or decades	Average number of vertebrae in offspring
112	135	112.948
111	105	112.523
110	47	111.596
109	11	111.245
108	7	110.400
107	2	109.250

Table V. — Transplantation of population samples from Station 31 and Station 34, 1916. — Decade investigations.

	Station 31	Station 34
Average number of vertebrae in offspring of transplanted sample	**114.819 ± 1.059** (27 decades)	**108.283 ± 1.363** (11 decades)
Average number of vertebrae in transplanted mothers	113.115 (26 specimens)	107.00 (9 specimens)

Table VI. — Station 31, Nakkehage, Ise Fjord, Denmark. — Transplantation Experiments, 1916—1917. — Decade investigations.

Average number of vertebrae in offspring of transplanted specimens and in offspring developed under natural conditions.

Maternal number of vertebrae	Average number of vertebrae in offspring of Transplanted specimens	Not transplanted specimens
116	116.14 (7)	114.16 (65)
115	115.22 (17)	113.85 (109)
114	114.59 (18)	113.52 (153)
113	115.34 (17)	113.12 (185)
112	114.14 (18)	112.95 (135)
111	113.91 (22)	112.52 (105)
110	112.59 (9)	111.60 (47)

The figures in brackets indicate the number of mothers or decades.

Table VII. — The *Zoarces* populations of the Ise Fjord - Roskilde Fjord area. — Population analyses 1919.

	Average number of vertebrae.
Station 68, Kattegat (outside fjord)	117.330
— 69 (mouth of fjord)	114.563
— 70 (— - —)	113.732
— 31, Ise Fjord	113.2 (1914—19)
— 32, —	112.939
— 74, —	112.243
— 75, —	112.225
— 71, Roskilde Fjord	111.168
— 72, — —	110.892
— 73, — —	109.152
— 34, — —	108.0 (1914—19)

Carlsberg Laboratory, 22. February 1920.

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A PRACTICAL FORM OF THERMOSTAT AQUARIA HEATED BY ELECTRICITY.

BY

O. WINGE.

When investigating organisms living in water, thermostat aquaria are often necessary. This is true in genetic experiments with fish, if on the one hand the most homogenous conditions for the experimental fish possible are to be procured, and if on the other, animals are to breed in various degrees of temperature.

In the following is described a practical application of such a thermostat, used with satisfactory result for several years at the Carlsberg Laboratory, without any defects being discovered.

The framework of the aquarium proper is constructed of 1.5 mm. angle-bars. Plate glass is fitted into all four sides. The bottom consists of a thin sheet of iron. Each aquarium is 41 cm. in length, 24 cm. in breadth and 39 cm. in height, 10 cm. of which are subtracted for the legs. Under the bottom of the aquarium is a thin sheet of zinked iron, on which 5 electric heating resistances are placed in such a way that they are insulated (by means of asbestos) downwards, while heat is transmitted upwards through the iron bottom of the aquarium to the water. The radiators have a resistance of 260 ohms each and are placed lengthwise of the aquarium. In fig. 1 the are drawn too short, due to lack of space. By means of the switches, (see bottom of fig 1, a) the current can be cut off from both 1 and 2 radiators respectively, and the heat regulated at will. As the current passes through 5, 4, 3 or 2 radiators, the voltage being 220, its electromotiv power is 0.17, 0.21, 0.28 or 0.42 amperes, and about 37, 46, 62 and 92 watts, respectively, are used.

The thermo-regulator, which is submerged in one corner of the aquarium and fastened to a piece of metal, is of glass, filled with xylol and mercury, and has the form shown in fig. 1, t.

The current passing through the regulators is controlled automatically by the mercury, which at the temperature required in the aquarium closes a weak circuit through and electro-magnet[1]) (fig. 1,i,) which. in turn, by attracting its anchor, shuts off the main circuit.

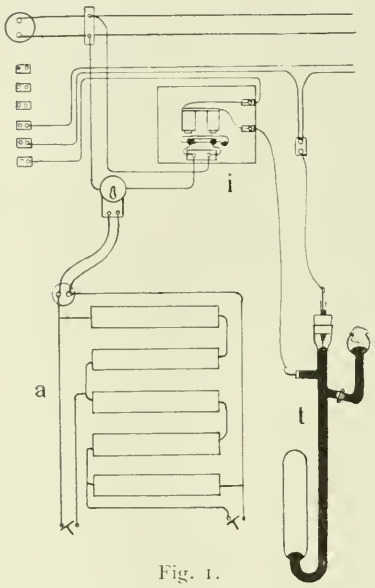

Fig. 1.

This anchor, through which the main circuit is closed, is enclosed in an horizontally-placed, sealed glass tube[2]) filled with a neutral gas. In this tube, which is 55 mm. long and 7 mm. thick, two platinum wires, connected with the electrodes of the main circuit, are fastened into two small globes, blown in the glass. Two drops of mercury, lying in the small globes, conduct the current from the platinum wire through the anchor, which, when the current is switched off, floats on the drops of mercury[3]). When the weak circuit is closed, the anchor is lifted by the electro-magnet and the main circuit is thus interrupted. When the temperature in the aquarium falls, the mercury in the termometer tube will sink and interrupt the weak circuit. When the anchor falls down on the drops of mercury, the main circuit is closed. The weak circuit with a voltage of about 2 is generated by help of a secundary battery of 6 cells[4]), which is charged from the 220 volt town main, 1—3 carbon filament lamps being inserted for obtaining a suitable resistance. 6 aquaria are placed in series. In fig. 2, however, one has been

[1]) From a dismounted electric bell.

[2]) It is not sufficient to create a vacuum in the tube, for if the tube is not filled with a neutral gas the iron will be corroded, and both the drops of mercury and the inside of the tube will be covered with iron dust, which is a poor conductor.

[3]) In addition to the globes already referred to, the glass tubes have a globe in one end in which the surplus of mercury is stored.

[4]) Ordinary primary batteries would very soon become exhausted.

removed. The electric wires and switches are attached to a vertical board placed over the aquaria, as shown on the figure.

The aquaria are ventilated with air by means of an electrically driven centrifugal pump. This automatically fills an iron bell, which, properly loaded, in its turn floats in an iron cylinder filled with water. Ventilation is not only necessary to many organisms but the circulation af air brings the water in motion

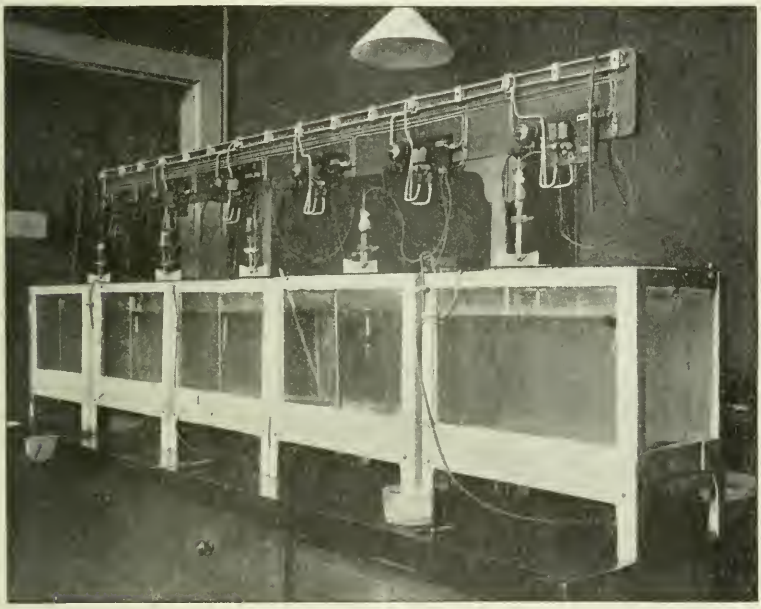

Fig. 2.

and the changes in temperature become minimal. When the thermometer-pipe of the regulator is narrow (about 1 mm.) the variations are commonly less then $^1/_{10}{}^0$. It is often advisable to cover the bottom of the aquarium with a layer of sand for the benefit of the animals used in the experiment. This layer of sand restrains the transmission of heat to the water, thus causing the variations to be greater. The layer of sand, if several cm. thick, will accumulate so much heat, that the temperature of the water, even after the current is switched off, will raise considerably, up till several tenths of a degree.

As the radiators are placed lengthwise under the bottom of the aquarium, this may, if desirable, be divided crosswise by a

sheet of glass without danger of the two parts of the aquarium becoming unequally heated.

If constant temperature, lower than the room is desired, one or two smaller aquaria may be placed inside the aquarium in question, and ice-water allowed to drip into this outer aquarium, where the regulator is placed.

The apparatus here described is very reliable, several months may pass without there being anything to repair. From time to time, however, the pipe of the thermo-regulator should be cleaned by means of a little cotton on a pin, and the accumulator cells should be charged once a year.

My thanks are due to Professor, Dr. Aug. Krogh for the suggestion about the switch.

Carlsberg Laboratorium, December 15, 1919.

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paraissent par livraisons à des époques indéterminées. A mesure qu'il en paraîtra un nombre suffisant pour faire un volume, les abonnés recevront un titre en même temps qu'une table des matières, avec l'indication de la période qu'embrasse le volume.

JOHS. SCHMIDT:

RACIAL INVESTIGATIONS.

VI. STATISTICAL INVESTIGATIONS ON INHERITANCE IN *ZOARCES VIVIPARUS* L.

BY

KIRSTINE SMITH.

I. Introduction.

The investigations that will be mentioned here are connected with two works by Dr. Johs. Schmidt on *Zoarces viviparus* published in "Racial Investigations" I and II[1]). In Section VI of the first of these papers the results of several individual offspring analyses were already given, but the material was not at that time sufficient for a closer investigation of inheritance. More material has since then been supplied with such an investigation in view. The material upon which the present paper is founded consists of samples of males and females, as a rule gravid, from Station Nakkehage 31 in Ise Fjord (in the northern part of the Danish island Sealand)[2]). The samples were taken in each of the years 1914—1919 incl. The chief results concerning inheritance of one of the characters dealt with here, viz. the number of vertebrae, have shortly been stated by Dr. Johs. Schmidt[3]), while Dr. Schmidt has asked me now to undertake the closer statistical analysis which is beyond the proper domain of a biologist.

The samples were collected by Capt. G. Hansen, who together with the attendant of the Laboratory Mr. N. R. Poulsen

[1]) Comptes Rendus des Travaux du Laboratoire de Carlsberg, Vol. **13**, Nr. 3, 1917, Vol. **14**, Nr. 1, 1917.

[2]) See "Racial Investigations" I, Pl. II, Station 31.

[3]) Johs. Schmidt: "Racial Investigations" V, Compt. Rend. des Trav. du Lab. de Carlsb. **14**, 1920. Johs. Schmidt: Racial Studies in Fishes, IV. Experimental investigations with *Zoarces viviparus* L. (Journal of Genetics, Vol. X, Nr. 3, 1920).

also undertook the preparation of the specimens, i. e. placing of serial numbers, skeletonization, and staining. Besides Dr. Johs. Schmidt several naturalists in particular Mr. Vilh. Ege, M. Sc., and Mr. A. Strubberg, M. Sc. have examined the material.

II. Material.

The investigations of inheritance in the case of *Zoarces* depend upon the fact, that it is viviparous, and that it is therefore possible to compare the mother and the offspring and the offspring mutually, when the collection of samples takes place at a season, when the females are gravid and the embryos well-developed. That is the case in the late autumn, and at this time of the year the samples were accordingly collected in the six years 1914—1919.

The characters examined are (1) number of vertebrae ("Vert"), (2) number of rays in the right and the left pectoral fin ("Pd" and "Ps"), and (3) number of pigment spots on the part of the dorsal fin consisting of soft rays. These characters can be accurately determined in the embryos, while the sex of these is unknown.

The number of specimens contained in each of the six population samples varies between 168 and 842, of these somewhat less than half are males. The number of vertebrae were counted for a total number of 857 gravid females. They often contain a great number of embryos, as many as 550 have been observed, but as a rule only ten embryos (a "decade") taken at random have been examined.

Dr. Johs. Schmidt has already in his first paper on *Zoarces* found that the characters dealt with here exhibit the same mean values in the two sexes. As the sex of the embryos is unknown, and as it is only possible to compare the offspring with the mother, this result is essential for the following investigations. It will therefore first be shown that this result is confirmed by the considerable amount of new material.

For each of the three characters in Table 1 are given the distributions of frequencies for males and for females, together with total number (n), mean value (a), presumptive standard deviation ($_p\sigma$)[1]), and probable error (p. e. (a)) of the mean. It is seen

[1] See formula and footnotes p. 6.

Table 1. — Comparison between Males and Females, Nakkehage 1914—1919.

Number of Vert	♂ Frequency	♀ Frequency	Number of pigment spots (D_1)*	♂ Frequency	♀ Frequency	Number of pect. fin rays (Pd)	♂ Frequency	♀ Frequency
119	4	4	20	1	1	21	6	10
118	10	12	19	3	1	20	263	375
117	23	40	18	6	7	19	547	709
116	72	85	17	17	20	18	66	103
115	127	139	16	61	48	17	3	»
114	161	217	15	113	155			
113	163	248	14	178	231			
112	134	185	13	139	188			
111	98	138	12	107	128			
110	48	67	11	34	43			
109	23	21	10	10	12			
108	5	12	9	3	2			
107	2	5	8	»	1			
106	»	»						
105	1	»						
104	»	1						
n	871	1174		672	837		885	1197
a	113.214	113.127		13.734	13.657		19.229	19.244
$p\sigma$	2.042	2.031		1.628	1.534		0.600	0.611
p. e. (a)	0.047	0.040		0.042	0.036		0.014	0.012

* Counting of pigment spots is only undertaken in the years 1915—1919.

from the table, that for the pectoral fins the difference between the mean values is smaller than the probable error of the difference, and for the two other characters the difference is smaller than 1.5 times its probable error. It can thus be considered well established that no difference of sex can be traced in the three characters as far as the means are concerned[1]).

The gravid females and their embryos supply the material for forming correlation tables (Tabl. 11—33) for mothers and offspring. As for each mother ten embryos were examined, the mother appears combined with each of these, i. e. ten times. The tables are given for each sample separately and for all the samples taken together for the vertebrae, for which character no essential deviations are found between the samples.

Each table yields material for calculation of the mean value and the standard deviation for mothers and for embryos, the coefficient of regression for offspring and the correlation coefficient between mothers and offspring. But it is not possible from these tables alone to calculate the probable error of the mean for embryos, of the coefficient of regression, nor of the correlation coefficient, because the embryos consist of groups of ten siblings[2]) between the values of which there is correlation. For this reason the usual formulae for these probable errors, which suppose that the observations of each of the characters mutually have the correlation coefficient zero, cannot be used.

As a supplement to the correlation tables and as useful for the calculation of probable errors just referred to Tables 34—37 are provided. They give approximately or exactly the mean values (mean values of "decades") for each group of siblings. At the foot of the tables are given the squared standard deviations of the mean values for decades, directly calculated from these mean values. From these standard deviations is calculated the probable error of the mean for the total number of embryos contained in a sample (Tabl. 2—5). The standard deviation of means of decades

[1]) As to the variation of the characters it is however possible that there is for pigment spots a small difference, so that the males vary more than the females. The difference between the values of $_p\sigma$ is 0.094, i. e. 2·4 times the p. e., and an examination of the individual samples shows for four cases out of five a deviation in the same direction.

[2]) The word "sibling" (Anglo-Saxon) was introduced by Pearson (Roy. Soc. Proc., Vol. 66, p. 157) for "either member of a pair of offspring from the same parent."

is further used for calculation of the fraternal coefficient of correlation, through which the laborious construction of fraternal correlation tables is avoided.

III. Statistical Methods and Formulae.

A. General Survey.

As it will be shown in a later section the regression curves, representing the mean values for offspring for given values of mothers, are linear for the characters dealt with here. As it will also be shown later on, the distribution of frequencies for mothers and for offspring may with a close approximation be represented by the normal law of frequency. The variation of the two generations and the relation between mothers and offspring will therefore be fully described by stating the mean values, standard deviations, and the regression or the correlation coefficient (the "maternal"). The generally used formulae and methods of calculation for determining the correlation will thus provide what is needed for treating the material except as regards some of the probable errors, in the calculation of which we have to remember that the embryos consist of groups of ten siblings between the values of which there is correlation.

In the determination of correlation between siblings ("fraternal" corr.) the constant number of siblings provided us with the opportunity of using an easy method of calculation, which makes the laborious construction of fraternal correlation tables unnecessary, but which at the same time, it is true, excludes the analysis of the form of the regression curve.

A list will first be given of the notations and formulae, and then the special formulae for standard deviations and fraternal correlation will be deduced.

Notations and Formulae.

Individual values for mothers x
Individual values for offspring ..... y
or for individuals of the p^{th} decade .. $y_{p,1}$, $y_{p,2}$ ..... $y_{p,10}$
with mean value..... y_p
Number of mothers..... n
Number of embryos ..... $10n$
Mean value for mothers ..... $a_1 = \frac{\Sigma x}{n}$

Mean value for embryos ..... $a_2 = \frac{\Sigma y}{10\,n}$

Standard deviation for mothers ..... $\sigma_1 = \sqrt{\frac{\Sigma (x - a_1)^2}{n}}$

Presumptive standard deviation[1]) ..... ${}_p\sigma_1 = \sqrt{\frac{n}{n-1} \cdot \sigma_1{}^2}$)

Standard deviation for embryos ..... $\sigma_2 = \sqrt{\frac{\Sigma (y - a_2)^2}{10\,n}}$

Standard deviation for means of decades .. $\sigma_d = \sqrt{\frac{\Sigma (y_p - a_2)^2}{n}}$

Regression coefficient ..... $\alpha = \frac{\Sigma (x - a_1)(y - a_2)}{10\,n \cdot \sigma_1{}^2}$

Maternal correlation coefficient ..... $r_m = \frac{\Sigma (x - a_1)(y - a_2)}{10\,n \cdot \sigma_1 \cdot \sigma_2}$

Fraternal correlation coefficient

$$r_f = \frac{S\Sigma (y_{p,1} - a_2)(y_{p,2} - a_2)}{45\,n \cdot \sigma_2{}^2} = \frac{1}{9}\left(\frac{10\,\sigma_d{}^2}{\sigma_2{}^2} - 1\right)$$

Probable error of a_1 ..... p. e. $(a_1) = \frac{0.67449}{\sqrt{n-1}}\,\sigma_1$

— — - a_2 ..... p. e. $(a_2) = \frac{0.67449}{\sqrt{n-1}}\,\sigma_d$

— — - α ..... p. e. $(\alpha) = \frac{0.67449}{\sqrt{n-1}} \cdot \frac{\sigma_d}{\sigma_1} \sqrt{1 - r_m{}^2 \frac{\sigma_2{}^2}{\sigma_d{}^2}}$

B. Special Formulae for Standard Deviations.

The above mentioned difficulty in determining the standard deviation of functions of values ($y_{p,1}$) of the embryos is easily overcome, when these individual values can be replaced by mean values (y_p) for siblings. Tables of y_p and their squared standard deviations, $\sigma_d{}^2$, (Tabl. 34—37) is all that is needed. This applies of course in the first instance to the mean of all embryos, which can be regarded as the mean of the mean values of the decades, and for the probable error of which the above simple expression by σ_d is therefore found.

---

[1]) In the publications of the Laboratory for a standard deviation is usually given the presumptive st. d. As in the section on fraternal correlation a clear distinction is wanted between actual and presumptive st. d. a special notation is introduced here.

[2]) See T. N. Thiele: Theory of Observations, London 1903, p. 48.

In the same way the slope of the regression line of offspring on mothers can as well be calculated from the mean values of decades as from the individual values for offspring When the slope, a, is regarded as a function of the means of decades, which can be considered as independent observations, the usual formula for the st. d. of the slope can straightaway be applied.

It is

$$\sigma_a = \frac{\sigma_d}{\sqrt{n-1}\,\sigma_1}\sqrt{1-r_d^2}\,^{1)},$$

where r_d is the correlation coefficient between mothers and the means of decades. For r_d by means of the correlation coefficient (r_p) between mothers and individual embryos we evidently have the expression

$$r_d = r_p \frac{\sigma_2}{\sigma_d}.$$

When this value is substituted for r_d in the formula above, we find the probable error of a as given in the above list

$$\text{p. e. } (a) = \frac{0.67449}{\sqrt{n-1}} \cdot \frac{\sigma_d}{\sigma_1}\sqrt{1-r_p^2\,\frac{\sigma_2^2}{\sigma_d^2}}.$$

For neither the st. d. of the maternal nor of the fraternal correlation coefficient have I at present formulae ready, i. e. formulae which can be used when so great a number as ten siblings are employed for the calculation. When all values of each of the variables are mutually independent the following formula,

$$r = \frac{1-r^2}{\sqrt{N-1}}\,^{2)}$$

holds for the standard deviation of the correlation coefficient, r, calculated from N pairs of the two variables. For the standard deviation of the maternal correlation coefficient a lower and higher limit is therefore obtained by putting N equal to 10 n and n, but a better approximation is reached by taking the st. d. of the slope of the regression line[3]).

For the st. d. of the fraternal correlation coefficient a lower limit is obtained by putting N equal to 45 n, i. e the number of

[1] Pearson and Filon: Phil. Trans. Vol. **191** A, p. 245, 1898.

[2] See Pearson and Filon: l. c., p. 229, and H. E. Soper: On the probable error of the correlation coefficient. Biometrika, Vol. IX, p. 107, 1913.

[3] See pp. 23—24.

examined pairs, and a higher limit is determined by N = n, by which only one pair of siblings from each family is taken into consideration. These limits are however so wide that they are of small practical consequence, and the accuracy of the fraternal correlation coefficient must be judged by comparing the results obtained from the various samples.

C. Fraternal Correlation.

To deduce the formula for the fraternal correlation coefficient we start from the equation

$$10\ y_p = y_{p,1} + y_{p,2} + \ldots + y_{p,10},$$

which expresses that the sum of the values for ten siblings is equal to ten times their mean value. By the subtraction of 10 × the mean value of all embryos from each side of the equation and squaring we find:

$$100\ (y_p - a_2)^2 = \Sigma (y_{p,1} - a_2)^2 + 2\,\Sigma (y_{p,1} - a_2)\,(y_{p,2} - a_2),$$

which by summation for all groups of siblings and division by 10 n leads to

$$10\,\sigma_d{}^2 = \sigma_2{}^2 + \frac{1}{5\,n}\,S\,\Sigma (y_{p,1} - a_2)\,(y_{p,2} - a_2).$$

The fraternal correlation coefficient is however by definition

$$r_f = \frac{S\,\Sigma (y_{p,1} - a_2)\,(y_{p,2} - a_2)}{45\ n\ \sigma_2{}^2},$$

from which follows

$$r_f = \tfrac{1}{9}\left(\frac{10\,\sigma_d{}^2}{\sigma_2{}^2} - 1\right).$$

This formula, which I have used for calculation of the fraternal correlation coefficient, requires besides the st. d. for offspring only the st. d. for means of decades. If we imagine siblings identical, σ_d does not differ from σ_2, and the formula gives $r_f = 1$, as it ought to. If on the contrary siblings are supposed to differ as much from each others as unrelated individuals, we have $\sigma_d{}^2 = \frac{1}{10}\,\sigma_2{}^2$, because the means of decades simply differ from each other as do the means of ten values for embryos taken at random. We therefore find $r_f = 0$, in accordance with the supposition.

IV. Variation and Maternal Correlation.

A. The Shape of the Regression Curve.

The regression curves do not show any essential deviation from a straight line, so that the inheritance with close approxi-

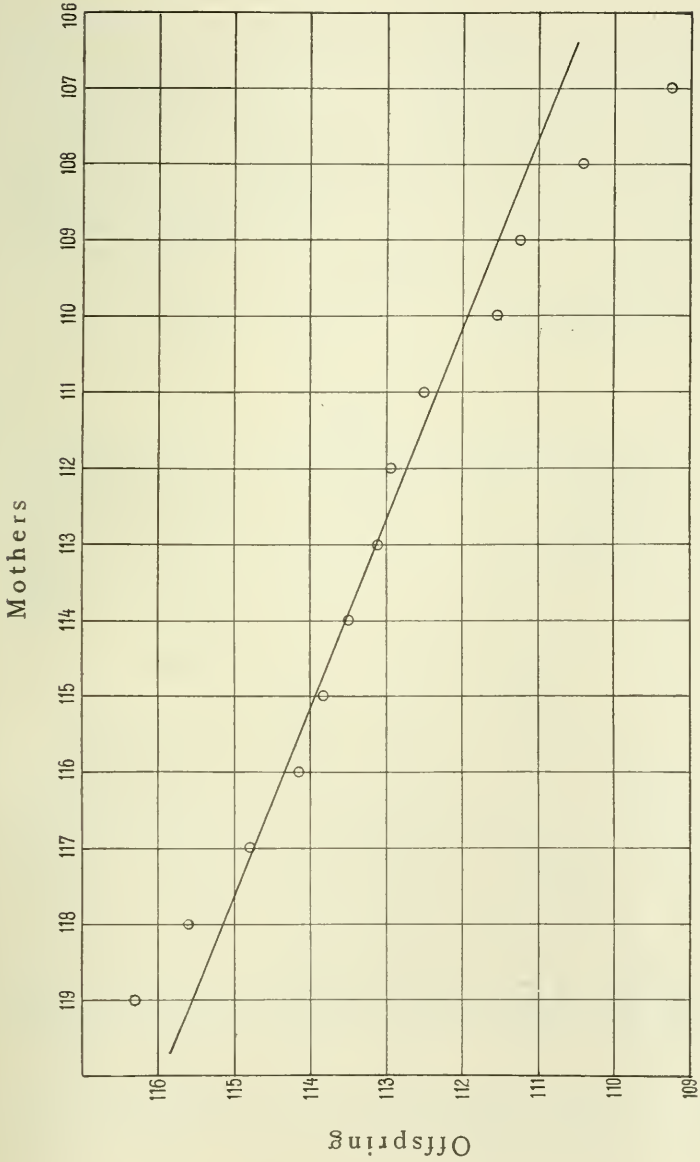


Fig. 1. *Probable Number of Vertebrae in Offspring for given Number of Vertebrae in Mother.*
Regression Line: $y = 67{,}452 + 0.4043\,x$; 8570 Embryos 1914—1919.

mation can be described by the correlation coefficient. As examples three regression lines will here be examined, all of them representing regression of offspring on mothers:

Fig. 1. Vert. 1914—1919,
Fig. 2. Pd 1917 and
Fig. 3. Pigm. D_1 1917.

It will be seen from Fig. 1, that the means of offspring from mothers with values from 109 to 117 are grouped rather closely about the regression line, while the two extreme values at each end deviate. These arrays arise however only from the offspring of a total number of 18 (= 2 + 7 + 6 + 3) mothers, so that it cannot be expected that the paternal inheritance for these mean values approaches the general mean value of the number of vertebrae. A closer judgement of the significance of these deviations is obtained by a determination of the correlation ratio, η, which is by Pearson defined by

$$\eta^2 = \frac{S\, n_x (y_x - a_2)^2}{N\, \sigma_2{}^2},$$

where n_x is the number of y corresponding to a given x, and y their mean, while N is the total number of pairs of observations. Pearson has shown[1]), that the necessary and sufficient condition for linear regression is $\eta^2 - r^2 = 0$. For the st. d. of $\eta^2 - r^2 = \zeta$ John Blakeman[2]) has given the following approximate formula:

$$\tfrac{1}{4}\, \sigma_\zeta{}^2 = \frac{\eta^2 - r^2}{N} \{(1 - \eta^2)^2 - (1 - r^2)^2 + 1\}.$$

We find $\eta = 0.4093$, $r = 0.3981$ og $\eta^2 - r^2 = 0.0091$.

When calculating the st. d. we face the same difficulty as was mentioned in the preceding section, that the values of one of the variables are mutually correlated. The formula above can therefore only provide the higher and lower limits found by putting $N = n$ and $N = 10\,n$. In this way is found for $n = 857$:

$$0.0020 < \sigma_\zeta < 0.0065.$$

A comparison of the probable error of means for mothers (0.045) and of means for offspring (0.033) in Table 2 shows since the st. d.

[1]) Karl Pearson: On the general theory of skew correlation and non-linear regression. Drapers' Company Research Memoires. Biometric Series II.

[2]) Biometrika **4**, p. 339, 1905.

of individuals belonging to the two generations is approximately the same, that values for the 10 n embryos here only count as abt. 2 n independent measurements. It may therefore be supposed that the 10 n embryos also by calculation of $\eta^2 - r^2$ yield about the same accuracy as 2 n mutually unrelated embryos.

With this approximation is found:

$$\sigma_\zeta = \frac{0.0065}{\sqrt{2}} = 0.0046.$$

The value found for $\eta^2 - r^2$ is according to this about twice its probable error. There is thus a faint possibility of a real deviation from linear regression, but it is at any rate too doubtful to deserve further attention.

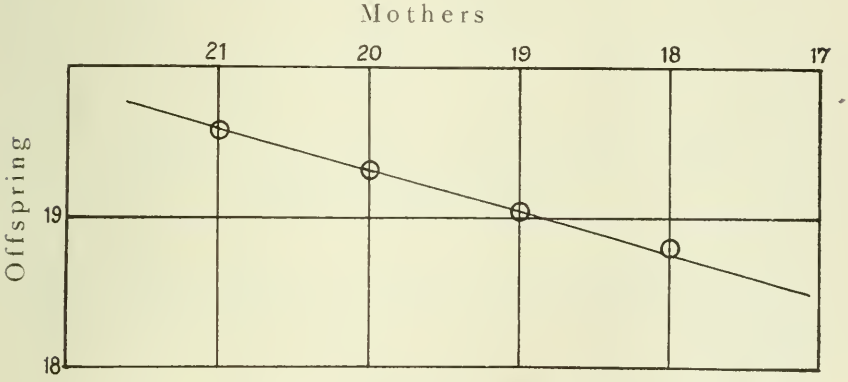


Fig. 2. *Probable Number of Pectoral Fin Rays, Pd, in Offspring for given Number in Mother.*

Regression Line: $y = 13.890 + 0.2708\,x$; 1760 Embryos from 1917.

The number of rays in the pectoral fins varies so much from year to year that it has not been possible to treat the samples all clubbed together, but only separately. In 1917 the largest sample occurs, and this has been chosen for illustrating the regression line. In Fig. 2 the means for offspring crowd so closely about the regression line as to convince directly of the goodness of the approximation. It also appears after calculation by means of the formulae above, that $\eta = 0.2839$ only differs very little from $r = 0.2824$.

For the st. d. of $\eta^2 - r^2 = 0.0008$ is found $\sigma_\zeta = 0.0014$, when the number of observed pairs is put equal to the numher

of embryos, by which a lower limit is obtained for the st. d. The value found for $\eta^2 - r^2$ is therefore less than half its standard deviation, so that the regression is decidedly linear.

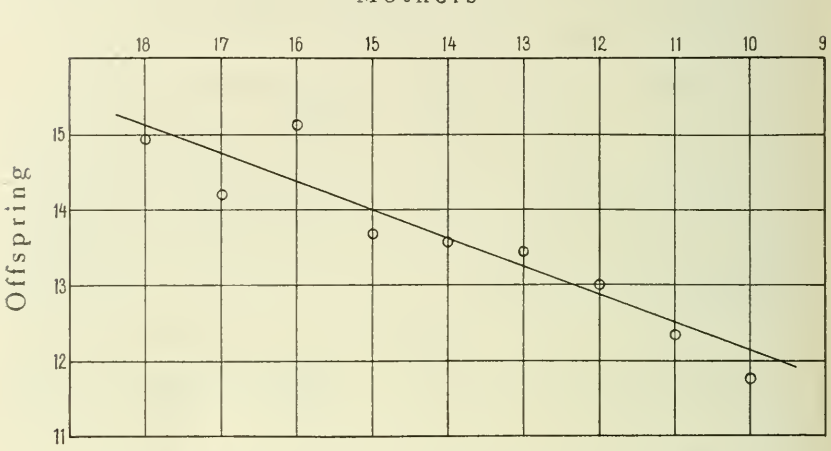


Fig. 3. *Probable Number of Pigment Spots in Offspring for given Number in Mother.*
Regression Line: $y = 8.386 + 0.3741\,x$; 1270 Embryos from 1917.

The regression line for number of pigment spots is represented in Fig. 3 for the sample of 1917. The following values are found:

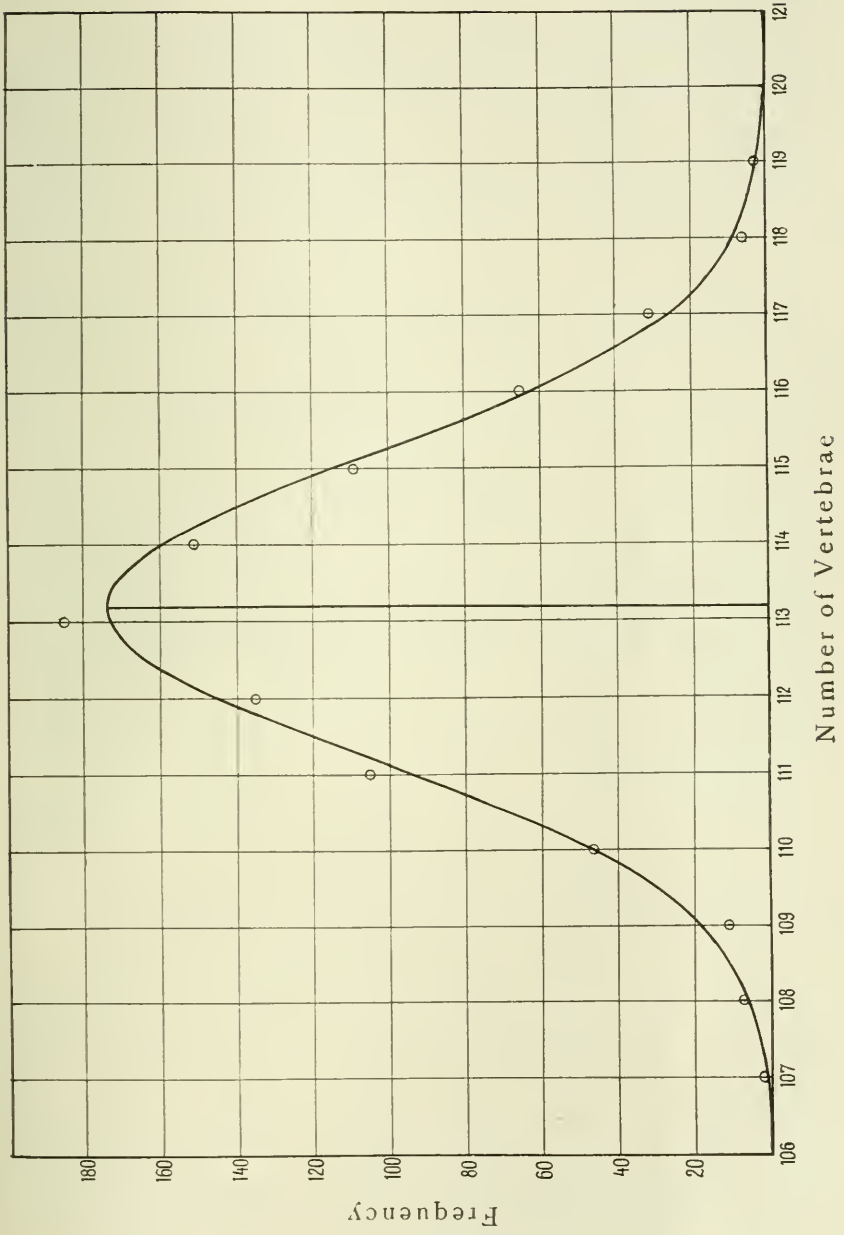
$$\eta = 0.4095,\ r = 0.3722 \text{ og } \eta^2 - r^2 = 0.0292.$$

For the st. d. of this difference are found, by first putting the number of examined pairs equal to the number of mothers and then equal to the number of embryos, the limits 0.0295 and 0.0093. Introducing for N as in the above example concerning vertebrae twice the number of mothers we find 0.0209. This value of $\eta^2 - r^2$ is thus only about twice its probable error, so that we are justified in considering the regression curve linear.

B. Distributions of Frequencies.

Both for the number of vertebrae and the number of pectoral fin rays the distribution of frequencies can with close approximation be represented by the normal law of frequencies. In Fig. 4 are shown

Fig. 4. *Distribution of Number of Vertebrae for 857 Mothers.*



the observed frequencies of number of vertebrae in mothers together with the normal law of frequency which best satisfies the frequencies.

The numerical values are given in the following table:

Number of Vertebrae in Mothers, $a_1 = 113.1855$, $\sigma_1 = 1.96156$.

	119 and over	118	117	116	115	114	113	112	111	110	109	108	107 and under
Observ. Freq.. .	3	6	31	65	109	151	185	135	105	47	11	7	2
Calc. Freq.. .	2.55	8.57	26.31	62.27	113.63	159.90	173.52	145.20	93.70	46.62	17.89	5.29	1.42
Obs.—calc. Freq.. .	+0.45	−2.57	+4.69	+2.73	−4.63	−8.90	+11.48	−10.20	+11.30	+0.38	−6.89	+1.71	+0.58

The agreement seems quite satisfactory, the deviations are relatively small and their signs changing. By using Pearsons criterion for goodness of fit[1]), the aim of which is to calculate the probability, P, that by random sampling from the same population as large or larger deviation between theory and observation than that in question occurs, the value 0.72 is found for P. It thus follows that taking 100 random samples, we must expect to find 28 agreeing better with the theory than our sample above, and 72 with greater deviation. The agreement must therefore be considered very good.

For number of pectoral fin rays in mothers 1917 it also appears that the normal law represents the frequencies well, as it will be seen from the following table:

Number of Pectoral Fin Rays, Pd, in Mothers 1917
$a_1 = 19.250$, $\sigma_1 = 0.60771$.

	21 and over	20	19	18 and under
Observ. Freq.	3	51	109	13
Calc. Freq.	1.832	53.95	106.16	14,054
Obs. — calc. Freq. . .	+ 1.168	— 2.95	+ 2.84	— 1.054

[1] Karl Pearson: Tables for Statisticians and Biometricians. London 1914, p. XXXI.

For the probability P is found the value 0.79, the distribution of frequencies therefore closely follows the typical law.

In counting the number of pigment spots the difficulty is met with that two spots happen to be partly connected, so that it is doubtful, whether they should be counted as one or two. It also frequently happens that a spot occurs just at the limit of the incurvated part, so that it is doubtful, whether it should be counted or not. Examining the embryos, individuals with such doubtful number of pigment spots have been discarded, as it was always possible to procure 10 siblings for which the number of spots was certain. For the mothers the uncertain countings are left out from this analysis, which therefore only concerns 488 mothers, while the offspring of the 209 "uncertain" mothers is also examined. The number of these mothers are for the years 1915—1919 respectively 48, 30, 29, 25 and 11 % of the total number of examined mothers, from which must be supposed, that the judgment, as is perhaps not surprising, has altered somewhat in the course of years. I shall now examine, whether owing to this discarding of mothers a selection has taken place with regard to the number of pigment spots, as it would seem plausible that doubtful countings especially occurred at individuals with numerous spots. If such a selection has taken place it must appear in a difference between the offspring group A, of the 488 mothers with certain numbers of spots, and the offspring group B, of the remaining 209 mothers. Calculated per 1000 individuals the frequencies of pigment spots of the two groups are distributed as shown in the following table:

Number of Pigment Spots in Embryos.

	20	19	18	17	16	15	14	13	12	11	10	9	8
Freq. in A-group . . .	0.2	1.6	6.8	21	67	147	249	260	156	65	20	6	0.8
Freq. in B-group . . .	0	2.9	7.2	31	64	169	245	248	140	61	17	6	0.5
Difference of Freq. . . .	+0.2	−1.3	−0.4	−10	+3	−22	+4	+12	+16	+4	+3	0	+0.3

It will be seen that numbers of spots greater than 14 occur more frequently in B-group than in A-group. Clubbing together

all frequencies of numbers of spots greater than 14 the difference between the frequencies for the two groups is 30.5. The probable error of this number would for observation with correlation 0 be 7.8. As the squared standard deviation of the means of decades (vide Tables 37 and 5) is about half as large as the standard deviation of individual values for offspring, n groups, each consisting of 10 siblings, only gives the same accuracy for the frequencies per 1000 as 2n unrelated individuals would yield. It therefore follows that the above mentioned probable error really is about 7.8×1.5 or abt. 17. A similar result is reached by comparing the mean values for the two groups of offspring; they are 13.501 and 13.622. From Table 37 it will be seen that the squared st. d. of the means of decades for all samples is abt. 1.0. Using this value in the calculation, the probable errors of the two means are 0.031 and 0.047, and the p. e. of their difference, 0.121, is therefore 0.056. We have thus found, also for the means, a deviation abt. twice the p. e. If a selection has taken place by discarding the mothers, it is not so marked as to be certainly established by our examination of the offspring.

We shall now investigate whether the distribution of frequencies for the number of pigment spots in mothers for the greatest sample at hand, viz. that from 1917, follows the normal law. The observed and calculated frequencies with their deviations are given in the following table:

Number of Pigment Spots in Mothers 1917

$a_1 = 13.6772$, $\sigma_1 = 1.61627$.

	18 and over	17	16	15	14	13	12	11	10 and under
Observ. Freq. . . .	2	4	9	20	37	26	16	11	2
Calc. Freq. . . .	1.030	3.79	11.16	22.42	30.73	28.71	18.30	7.95	2.904
Obs. — calc. Freq. . . .	+0.970	+0.21	−2.16	−2.42	+6.27	−2.71	−2.30	+3.05	−0.904

Pearson's criterion for goodness of fit gives $P = 0.77$, so that also this character follows the normal law.

C. Comparison of the Mean Values for Different Samples and for the Two Generations.

In the following four tables is given the material for testing the stability of the population, of the variation between mothers and between offspring, and of the maternal correlation.

Table 2. — Vert. Variation and Correlation between Mothers and Offspring.

Year	n Numb. of Mothers	$a_1 \pm$ p. e. (a_1) Mean for Mothers $\pm$ p. e.	$a_2 \pm$ p. e. (a_2) Mean for Offspring $\pm$ p. e.	σ_1 St. D. for Mothers	σ_2 St. D. for Offspring	$\alpha \pm$ p. e. (α) Regression Coefficient $\pm$ p. e.	r_m Maternal Correlation Coefficient
1914...	138	113.536 ± 0.107	113.184 ± 0.084	1.85003	2.02676	0.3849 ± 0.0394	0.3513
1915...	168	113.161 ± 0.103	113.264 ± 0.076	1.97101	2.02370	0.4492 ± 0.0308	0.4375
1916...	123	113.276 ± 0.127	113.139 ± 0.089	2.08479	1.94628	0.3864 ± 0.0355	0.4139
1917...	177	113.102 ± 0.093	113.248 ± 0.075	1.83834	2.03502	0.4178 ± 0.0348	0.3775
1918...	153	113.039 ± 0.113	113.249 ± 0.079	2.06395	1.98065	0.4206 ± 0.0306	0.4382
1919...	98	113.000 ± 0.132	113.123 ± 0.088	1.92195	1.87575	0.3586 ± 0.0385	0.3674
1914—1919	857	113.186 ± 0.045	113.211 ± 0.033	1.96156	1.99219	0.4043 ± 0.0141	0.3981

Table 3. — Pd. Variation and Correlation between Mothers and Offspring.

Year	n Numb. of Mothers	$a_1 \pm$ p. e. (a_1) Mean for Mothers $\pm$ p. e.	$a_2 \pm$ p. e. (a_2) Mean for Offspring $\pm$ p. e.	σ_1 St. D. for Mothers	σ_2 St. D. for Offspring	$\alpha \pm$ p. e. (α) Regression Coefficient $\pm$ p. e.	r_m Maternal Correlation Coefficient
1914.....	132	19.462 ± 0.033	19.280 ± 0.022	0.56950	0.59914	0.2534 ± 0.0355	0.2409
1915.....	174	19.264 ± 0.030	19.326 ± 0.022	0.58644	0.61522	0.3373 ± 0.0329	0.3215
1916.....	122	19.270 ± 0.037	19.025 ± 0.024	0.60105	0,58409	0.2056 ± 0.0383	0.2116
1917.....	176	19.250 ± 0.031	19.102 ± 0.019	0.60771	0.58269	0.2708 ± 0.0288	0.2824
1918.....	156	19.231 ± 0.033	19.290 ± 0.020	0.60813	0.58477	0.2816 ± 0.0292	0.2928
1919.....	98	19.163 ± 0.047	19.450 ± 0.024	0.68054	0.59122	0.1608 ± 0.0343	0.1851
Average of annual values with weights $\frac{1}{\{\text{p. e. } (\alpha)\}^2}$						0.2570 ± 0.0133	0.2618

Table 4. — Ps. Variation and Correlation between Mothers and Offspring.

Year*	n Numb. of Mothers	$a_1 \pm$ p. e. (a_1) Mean for Mothers $\pm$ p. e.	$a_2 \pm$ p. e. (a_2) Mean for Offspring $\pm$ p. e.	σ_1 St. D. for Mothers	σ_2 St. D. for Offspring	$a \pm$ p. e. (a) Regression Coefficient $\pm$ p. e.	r_m Maternal Correlation Coefficient
1914.....	129	19.481 ± 0.034	19.323 ± 0.023	0.57196	0.58813	0.2431 ± 0.0368	0.2364
1916.....	121	19.273 ± 0.035	19.066 ± 0.023	0.57496	0.57546	0.1880 ± 0.0391	0.1878
1917.....	175	19.309 ± 0.029	19.154 ± 0.020	0.56233	0.58180	0.3139 ± 0.0311	0.3034
1918.....	156	19.244 ± 0.034	19.360 ± 0.020	0.63419	0.58263	0.2616 ± 0.0282	0.2847
1919.....	98	19.255 ± 0.045	19.477 ± 0.023	0.65949	0.57006	0.1663 ± 0.0327	0.1924
Average of annual values with weights $\frac{1}{\{\text{p. e. } (a)\}^2}$						0.2406 ± 0.0147	0.2487

* For the sample from 1915 the informations needed for a correlation table are not at hand.

Table 5. — Pigm. D_1. Variation and Correlation between Mothers and Offspring.

Year	n Numb. of Mothers	$a_1 \pm$ p. e. (a_1) Mean for Mothers $\pm$ p. e.	$a_2 \pm$ p. e. (a_2) Mean for Offspring $\pm$ p. e.	σ_1 St. D. for Mothers	σ_2 St. D. for Offspring	$\alpha \pm$ p. e. (α) Regression Coefficient $\pm$ p. e.	r_m Maternal Correlation Coefficient
1915.....	75	13.640 ± 0.121	13.657 ± 0.072	1.53744	1.47826	0.3617 ± 0.0373	0.3762
1916.....	87	13.598 ± 0.104	13.659 ± 0.075	1.43407	1.62085	0.4094 ± 0.0432	0.3622
1917.....	127	13.677 ± 0.097	13.502 ± 0.067	1.61627	1.62450	0.3741 ± 0.0348	0.3722
1918.....	113	13.814 ± 0.101	13.300 ± 0.058	1.59949	1.48542	0.3445 ± 0.0288	0.3710
1919.....	86	13.895 ± 0.110	13.463 ± 0.077	1.50988	1.59862	0,3579 ± 0.0439	0.3380
Average of annual values with weights $\frac{1}{\{\text{p. e. } (\alpha)\}^2}$						0.3651 ± 0.0162	0.3665

It will be seen from Table 2, that the means of number of vertebrae in embryos are essentially constant for the various samples, the greatest difference (between 1915 and 1919) being 0.141 with p. e. 0.116. The means for mothers in the five samples 1915—1919 also approach one another closely while that for 1914 is somewhat greater, so that the difference between the values for 1914 and 1919 is 0.536 with p. e. 0.170. Comparing mothers and their offspring, the difference is in three cases found to be below the p. e., and the greatest deviation is only 2.6 times the p. e. Taken as a whole the result is, that the number of vertebrae keeps constant for mothers and offspring and that, as far as concerns this character, the examined individuals may be considered as one sample.

For the number of pectoral fin rays (Table 3 and 4) it is observed that the means for Ps are always a little greater than for the corresponding Pd. The difference is for the five samples 1914 and 1916—1919, when both mothers and offspring are considered on a average only 0.042 with p. e. 0.013, but as Ps in all the ten cases has a greater mean than Pd, the difference is without doubt real. For a single sample the difference between the means for Ps and Pd is on an average about the size of its p. e. As the deviations between various samples and between mothers and their offspring, as it will be seen, is much greater it will suffice in the following to regard Pd alone.

The differences between means for mothers in the five samples 1915—1919 keep below twice their p. e., the 1914-sample however has a much greater mean, which deviates by 0.299 ± 0.057 from the smallest of the other means. The means for embryos deviate still more decidedly among themselves, there being differences between them a great as 0.425 with p. e. 0.034. Comparing mothers with their offspring, the greatest difference is 0.287 with p. e. 0.053. It is thus obvious that the number of pectoral fin rays within the same populaton is a much more variable character than the number of vertebrae. As the means for the embryos is sometimes greater and sometimes smaller that those for their mothers and as they vary strongly from year to year, notwithstanding that the means for the mothers are relatively constant, it must be assumed that the number of pectoral fin rays are much influenced by the physical conditions, which differ from year to year, for instance the temperature. Such a

varying influence must be expected to be distinctly traced in the means for single annual groups, as the embryos, while it is obscured in the means for mothers, consisting of fish of different ages.

The means of the number of pigment spots in mothers are essentially the same in the five samples, the greatest deviation being 0.297 with p. e. 0.151. Among the means for embryos that for 1918 is somewhat apart from the others which do not deviate essentially from each other. The number of spots for the 1918-sample was however counted after about six months' delay, and the spots were therefore much discoloured, so that the accuracy probably is somewhat reduced. It is therefore doubtful, whether there is a real difference. For the samples 1915—1917 the deviations between the means for mothers and their offspring are smaller than twice their p. e., in two cases the values are greatest for the embryos, and in the third for the mothers. For 1918 and 1919 the means for mothers are greater than for their offspring, in 1918, when the countings, as mentioned above, probably were less certain than usually, the difference is 0.514 with p. e. 0.116, and in 1919 0.432 with p. e. 0.134. Although less variable from year to year than the number of pectoral fin rays, the number of pigment spots thus appears to be a less constant character than the number of vertebrae. Possibly the observed deviations partly originate from the difficulties mentioned, which admit of the countings to be influenced by individual judgement. The counting was, it is true, in all the years carried out by the same person, and great care was taken, but as the number of discarded females varies from 48 to 11%, it is likely that it has not been possible to maintain a quite constant judgement.

When the deviation for pigment spots in 1918 is excepted, it holds on the whole for the three characters, that sometimes the offspring has the greater mean value and sometimes the mothers, the differences being not very essential. This affords a statistical confirmation of the fact that these characters are really determined and fixed in the embryos, as it has from a morphological point of view been concluded.

D. Maternal Correlation.

A comparison of the standard deviation for mothers and for offspring in Tables 2—5 shows that sometimes the mothers have the greatest st. d. and sometimes the embryos have the greatest st. d. There is no sign that the mothers form a selected less variable group of the population, and there has not been made any correction on account of selection of mothers. The correlation coefficients of the tables are therefore the raw values directly calculated from the correlation tables. As the st. d. for mothers and for offspring agree so closely, the regression coefficient and the correlation coefficient have nearly the same value, especially when the five or six samples are taken together. The reason why the regression coefficient is nevertheless given is that it is possible without difficulty to calculate the p. e. for the regression coefficient, while for the correlation coefficient I have had to content myself with approximate values for the p. e. as mentioned in Section III. For observations, where, for both of the variables the individual measures have no inter-correlation and where the st. d. has the same value for the two variables, the ratio of the st. d. of the correlation coefficient to the st. d. of the regression coefficient is $\sqrt{1 - r^2}$. Whether this holds, also when, as in the present case, one of the variables takes values between some of which there is correlation, I am not able to say at present. But it seems to appear from the numerical results that the two standard deviations are approximately equal. The various tables will now be gone through, so that the reader may judge the accuracy.

For the number of vertebrae the values of the regression coefficient agree well, the extreme values are 0.4492 and 0.3586 with the difference 0.0906 $\pm$ 0.0493. If the st. d. is calculated from the six annual values and from that the p. e. of the mean, which approximately coincides with the regression coefficient obtained for the six samples clubbed together, this p. e. is found to be 0.0089. In the corresponding way the p. e. of the correlation coefficient is found to be 0.0103.

It will be seen from Tables 3 and 4, when the values for a and r_m from the various years are compared, that small values for Pd are accompanied by small values for Ps, and great values for Pd by great values for Ps. The resemblance is so great that it will suffice to go through Table 3. The greatest deviation

between regression coefficients is 0.1765 with p. e. 0.0475. By direct calculation as above the p. e. of the mean of regression coefficients is found to be 0.0171, while from the p. e. for the various samples the p. e. of the average a is found to be 0.0133. The samples therefore differ a little more than was to be expected. For the p. e of the mean of the six r_m is by direct calculation found 0.0144.

The regression coefficients for pigment spots (Table 5) agree extremely well, the greatest deviation being 0.0382 with p. e. 0.0519. It also appears that the directly calculated p. e. of the mean of the five regression coefficients is 0.0075 while the theoretical p. e. is 0.0162. For the mean of correlation coefficients is found the p. e. 0.0050.

It seems to follow from this that the directly calculated p. e. of r_m is about as great as, or perhaps a trifle smaller than the directly calculated p. e. of a, and that the latter agrees quite well with the p. e. for a calculated by the formula. We therefore cannot be far from the truth when using these probable errors for r_m.

The main results for maternal correlation are gathered in the following table.

Table 6. — Maternal Correlation[1]).

Character	Numb. of vert.	Numb. of pect. fin rays (Pd)	Numb. of pigm. spots
Correlation Coefficient ± p. e.....	0.398 ± 0.014	0.262 ± 0.013	0.366 ± 0.016

It is evident from this that the maternal correlation is decidedly greater for the number of vertebrae and pigment spots than for the number of pectoral fin rays, a fact which is moreover confirmed when the correlation coefficient found for the various samples

[1]) As we have to do with discrete values for all the three characters, Sheppard's correction has not been made use of. In case than anybody should prefer to work with areas, the values found for r_m when Sheppard's correction is used for the calculation of standard deviations are given here. They are: Vert. 0.407, Pd 0.329 and Pigm. D_1 0.379.

are compared. It further appears that the maternal resemblance found, is somewhat smaller than that which is ordinarily found by investigations of so ample a material of observations for mammals. For physical characters in man and for the coat-colour in dogs and horses Pearson[1]) has found correlation coefficients abt. 0.5, and only for one character, forearm in man a value so small as 0.418, while the smallest value for the other characters is 0.459, found for the inheritance of span in man. The present material is, as regards number of examined pairs, solely inferior to one of those mentioned above, and also when it is taken into consideration that one mother with ten offspring individuals are less valuable for informations than two mothers with five children each, the present material is superior to the rest of the other series of observations. Besides, the characters dealt with here must be considered quite as well suited for accurate determination as characters which have to be measured, and moreover cannot be measured by one or a small number of persons. Therefore we consider on the whole, that the results of the present investigations as regards accuracy are on level terms with the results mentioned, and that it is therefore shown that the parental correlation coefficient for fish does not necessarily take a value close to 0.46, or between this value and 0.5, when sufficient accuracy is obtained, so as Pearson in the paper quoted, summarized the results then available on parental inheritance.

R. C. Punnett has by investigations of inheritance of various countable characters, among these the number of vertebrae, in *Spinax niger*[2]) arrived at values for parental correlation with average value 0.307 and limits 0.216 and 0.411, which falls outside the range of the present results. Punnett's material consisted of only 27 families; the probable errors of his results are therefore rather great. As so small values were not at that time found from materials of really satisfactory dimensions, there were reasons for regarding his results with doubt, and the author only claimed to have proved that inheritance was pointed out and did not ascribe the values found for the parental correlation coefficients

[1]) See K. Pearson and A. Lee: On the laws of inheritance in man. Biometrika, Vol. II. p. 379, 1903.

[2]) R. C. Punnett: Merism and Sex in *Spinax Niger*, Biometrika Vol. III. p. 344, 1905.

much more significance than that they were decidedly positive. Considered in connection with the present correlation coefficients the results found for *Spinax niger* appear much more probable.

V. Fraternal Correlation and Variation.

A. The Fraternal Correlation Coefficient.

The fraternal correlation coefficient is calculated from the formula:

$$r_f = \frac{1}{9}\left(10\,\frac{{\sigma_d}^2}{{\sigma_2}^2} - 1\right),$$

which is deduced in Section III. The numerical results are given in Table 7.

Table 7. — Fraternal Correlation.

Character	Vert.	Pd	Ps	Pigm. D_1
1914.....	0.4590	0.3169	0.3508	—
1915.....	0.4693	0.4196	—	0.3175
1916.....	0.5108	0.3985	0.3650	0.3418
1917.....	0.4715	0,3634	0.3742	0.4112
1918.....	0.4801	0 3329	0.3353	0.3074
1919.....	0.4066	0.2893	0,2698	0.3722
All Observ.[1]...	0.4695	0.3534	0.3390	0.3500
	± 0.0094	± 0.0137	± 0.0114	± 0.0128

For the probable error of the correlation coefficients a higher limit is obtained from the formula:

$$\text{p. e. } (r_f) = 0.67449\,\frac{1 - {r_f}^2}{\sqrt{n - 1}},$$

when n is put equal to the number of mothers, and therefore only one pair of siblings from each family is counted. In this way are found for the four character the values:

0.0180, 0.0207, 0.0229 and 0.0268.

[1] By using Sheppard's correction for discontinuity the following fraternal correlation coefficients are found: Vert. 0.480, Pd 0.420, Ps 0.451, Pigm. D_1 0.362.

Comparing with the probable errors of the means of correlation coefficients, directly calculated from the annual values, which are given in Table 7, it is seen that the ratio between corresponding values of the probable errors is nearly constant for the four characters and equal to 2. This supports the propable errors of the table and suggests that they are usable, although they are calculated from only 5 or 6 values. It further shows that 10 embryos from each of n mothers, with the present values of the fraternal correlation coefficient, determine this with about the same accuracy, as would be obtained from information about two embryos from each of 4 n mothers.

It is seen from Table 7 that the fraternal resemblance varies for the three characters; it is decidedly greater for vertebrae than for pectoral fin rays and pigment spots, for which two characters it is nearly equal. By comparison between Tables 6 and 7 it appears that the fraternal resemblance is greater than the maternal for the number of vertebrae, the difference between the two correlation coefficients being 0.071 with p. e. 0.017. The same holds for the pectoral fin rays, as the difference (for Pd) is 0.092 with p. e. 0.019. For pigment spots the correlation coefficients found are about equal.

For fraternal correlation even from ample materials widely varying values have been found. For various physical characters in man, correlation coefficients somewhat greater than 0.5 occur, and for coat-colour in dogs and thoroughbred horses as great values as 0.6—0.7 have been found[1]). As a rule the fraternal correlation is somewhat greater than the maternal, at any rate I do not know any case where it has been established that it was smaller than the parental. Compared with the parental correlation coefficients, the values for fraternal correlation are therefore not surprising.

For fraternal correlation in *Spinax niger* Punnett[2]) has found values from 0.254 to 0.447, with average value 0.374, which is about the size of our results. Prof. Punnett's material determines the fraternal correlation somewhat more accurately than

[1]) Pearson l. c., p. 390.
[2]) l. c., p. 342.

the maternal, but the material, consisting only of 27 mothers with a total number of 224 youngs, is in size much inferior to the present. No great stress could therefore be laid on a close coincidence of the results, but as both the investigations deal with fish and no other similar work, as far as I know, has been undertaken in that field, it is of great interest that the results in broad features resemble each other, especially as they differ from most of the results found for man and other mammals.

For neither of the investigations is it known to a certainty that the siblings examined are whole siblings. Punnett considered it the most probable for *Spinax niger*. Any decision on this question cannot be reached by Statistics, but various results arrived at concerning coat-colour in horse and cattle show that for this character the parental correlation coefficient lies between the correlation coefficients for half siblings and whole siblings. In an investigation by Barrington and Pearson[1]) on inheritance of coat-colour in shorthorns the following values were found: for parental correlation 0.46 and for correlation between whole and half siblings 0.53 and 0.32 respectively. If it be allowable to suppose that the law of inheritance for the present characters is analogous to the law found in that case, it would be expected that a fraternal correlation coefficient somewhat greater than or equal to the parental correlation coefficient, such as we have found it for the present characters, applies rather to whole than half siblings.

B. Fraternal Correlation — Fraternal Variation.

It is the aim of the following examination to determine to some extent whether measurements of a smaller number of groups of siblings may elucidate the question of fraternal correlation, and partly to provide material for judging, how far one has to proceed in the examining of siblings when an analysis of a great number of families is wanted, and it is therefore necessary to some extent to economise labour.

First an expression for fraternal correlation by means of fraternal variation will be sought. Using the notations given in

[1]) A. Barrington and K. Pearson: On the inheritance of coat-colour in cattle. Biometrika Vol. IV, pp. 448 and 454, 1906.

Section III, the sum of squared deviations for ten siblings from the total mean may be written:

$$\Sigma (y_{p.1} - a_2)^2 = \Sigma (y_{p,1} - y_p)^2 + 10 (a_2 - y_p)^2.$$

By taking the mean for all groups of siblings, we find from this:

$$\sigma_2{}^2 = \frac{1}{10n} S \Sigma (y_{p,1} - y_p)^2 + \sigma_d{}^2,$$

$\frac{1}{10} \Sigma (y_{p,1} - y_p)^2$ is the squared standard deviation for 10 siblings, and $\frac{1}{10n} S \Sigma (y_{p,1} - y_p)^2$ is the mean of such n squared standard deviations, which will be denoted by $\sigma_f{}^2{}_{,10}$. The presumptive st. d., i. e. the st. d. which we expect to find by calculation from an infinite number of siblings, is then:

$${}_p\sigma_f = \sqrt{\frac{10}{9}}\, \sigma_{f,10}.$$

By substitution in the equation found above is then seen that

$$\sigma_2{}^2 = \sigma_f{}^2{}_{,10} + \sigma_d{}^2$$

or

$$\sigma_2{}^2 = \frac{9}{10}\, {}_p\sigma_f + \sigma_d{}^2.$$

The formula for fraternal correlation was:

$$r_f = \frac{1}{9} \left(\frac{10\, \sigma_d{}^2}{\sigma_2{}^2} - 1 \right),$$

which by elimination of $\sigma_d{}^2$ is transformed to:

$$r_f = 1 - \frac{10\, \sigma_f{}^2{}_{,10}}{9\ \ \sigma_2{}^2}$$

or

$$r_f = 1 - \frac{{}_p\sigma^2{}_f}{\sigma_2{}^2} \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{I)}$$

It follows from this that if $\sigma_f{}^2$ was tolerably constant from one group of siblings to another it would be possible, by examining a few groups, each containing a great number of siblings, to obtain in an extremely easy way a determination of the fraternal correlation. In Table 8 are given the most important constants for the number of vertebrae for 12 great groups of siblings from Station Nakkehage[1]).

[1]) The distributions of frequencies of 8 of these samples are found in "Racial Investigations" I, p. 311.

Table 8. — Individual Offspring samples. Number of Vert. 1914.

Numb. of Vert. for Mother	Mean of Numb. of Vert. for Offspring	Squared Presumptive St. D. for Siblings $_p\sigma_f^2$	Numb. of Siblings
118	115.119	1.0764	67
110	111.106	1.2640	179
110	111.329	1.4544	79
112	112.360	1.9087	211
115	115.177	1.9586	203
118	116.338	2.0247	80
117	113.750	2.0897	40
117	113.923	2.3861	52
117	117.830	2.4869	94
108	109.483	2.6568	149
114	114.272	3.4995	184
113	112.365	4,2965	159

It will be seen that the standard deviations differ very considerably for different groups of siblings. The table is arranged according to increasing values of $_p\sigma_f^2$, which varies from 1.08 to 4.30. When analysing the table to find out the conditions for great values of $_p\sigma_f^2$, it is conspicuous that the two mothers the values, 113 and 114, of which approach the average for the population, have offspring with the greatest st. ds. As the means for the two groups of offspring also approach the average value for the population it must be assumed that this has also been the case for the values of the fathers. Regarding the smallest value of $_p\sigma_f^2$ it appears that the corresponding mother has the extreme value 118, while the father, judged from the mean of the value for offspring, must be supposed to have a value approaching the general mean for the population. The following small values of $_p\sigma_f^2$ occur in groups of siblings where evidently both the mother and the father have extreme values, deviating in the same direction. These facts seem to suggest that individuals with values near to the general mean of the population have a more complex gametal constitution than individuals with extreme values, so that the offspring of the former have possibilities of wider varia-

tion. The material is however too small for conclusive results, especially more samples are wanted from parents approaching the average, and there are also traces of disagreement with the rule pronounced, since both the third and fourth sample from the bottom seems to come from parents with extreme values, but nevertheless have rather great st. ds.

For all embryos from 1914 the value 4.1077 is found for $\sigma_2{}^2$, a value, which is smaller than that found for the last group of siblings. It is therefore evident that from a single or a few groups of siblings no reliable informations may be obtained of the fraternal correlation. It will be examined what result is reached by using the 12 groups of Table 8.

The mean of the 12 values of ${}_p\sigma_f{}^2$ is 2.2585 (with p. e. 0.1785), which substituted in (I) gives $r_f = 0.4309$. Reckoning that $\sigma_2{}^2$, as compared with the mean of ${}_p\sigma_f{}^2$ is without error, the p. e. of r_f is found to be 0.0434. In this way a value is arrived at for r_f, the accuracy of which is a good deal below the annual values of Table 7, the probable errors of these being

abt. $$0.0094 \sqrt{6} = 0.023,$$

and this is so, although a smaller number of embryos are employed for these determinations.

C. The Probable Error of the Mean Value for q Siblings.

Using the whole material of decades we found:

$$r_f = 1 - \frac{{}_p\sigma_f{}^2}{\sigma_2{}^2},$$

from which follows: $${}_p\sigma_f = \sigma_2 \sqrt{1 - r_f} \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(II)}$$

which determines the average standard deviation between a group of siblings. It was seen from Table 8 that the standard deviation for number of vertebrae in different groups of siblings could vary from abt. $\frac{1}{2}\,\sigma_2$ to σ_2, while (II) for the average st. d. gives $0.73\ \sigma_2$. If the knowledge of a group of siblings is restricted to a small number of individuals, the probable error of the mean for the various characters must be judged by means of (II). In the following table are given the probable error of means of number of vertebrae for 1, 2, 3 . . . and 10 siblings, the calculation being based upon (II).

Vertebrae. Probable Error for Mean Value for q Siblings.

Numb. of Siblings q	1	2	3	4	5	6	7	8	9	10
Prob. Error of Mean Value	0.979	0.692	0.565	0.489	0.438	0.399	0.370	0.346	0.326	0.309

D. Probable Error of the Mean for n Groups of q Siblings.

In the same way as the formula for fraternal correlation in Section III was deduced by means of decades, we find when q embryos from each mother are examined, and when σ_q denotes the standard deviations of their means:

$$r_f = \frac{1}{q-1}\left(q\frac{{\sigma_q}^2}{{\sigma_2}^2} - 1\right),$$

so that

$${\sigma_q}^2 = (1 + (q-1)\, r_f)\,\frac{{\sigma_2}^2}{q} \quad \ldots\ldots\ldots\ldots \text{(III)}$$

With increasing q, σ_q therefore approach:

$$\sigma_\infty = \sigma_2 \sqrt{r_f} \quad \ldots\ldots\ldots\ldots\ldots \text{(IV)}$$

From (III) it follows that the standard deviation, Σ, of the mean of nq embryos, offspring of n mothers, is determined by:

$$\Sigma^2 = (1 + (q-1)\, r_f)\,\frac{{\sigma_2}^2}{nq} \quad \ldots\ldots\ldots\ldots \text{(V)}$$

It is seen from this that to secure the same accuracy in the determination of that mean, as is obtained by 1000 unrelated embryos, 1000 $(1 + (q-1)\, r_f)$ embryos are required, when they consist of groups of q siblings. For number of vertebrae r_f is 0.4695, and it is found that, for determining the total mean for embryos, knowledge of 5226 embryos, consisting of groups of 10 siblings, has only the same value as knowledge of 1000 embryos from 1000 different mothers. In the following table are given the number of embryos which for values of q from 1 to 10 involve the same accuracy for the total mean.

Numb. of Siblings	1	2	3	4	5	6	7	8	9	10
Total Numb. of Embryos ..	1000	1470	1939	2409	2878	3348	3817	4287	4755	5226

If on the other hand one wants to know what can be gained by increasing the number of examined embryos, supposing the number of mothers constant, this question is elucidated by Table 9. The table contains the standard deviation of means of q siblings from 100 mothers, for varying numbers q, both calculated from (V) and calculated directly from means for groups of siblings. For this calculation the 1918-sample has been used, why also in (V) are introduced $r_f = 0.4801$ and $\sigma_2 = 1.98065$, which were found for that sample. In the first column of the table are found the serial numbers which have been given to the embryos in the decade, and in the last column the probable error of the mean is to be found.

Table 9. — Vert. 1918. Standard Deviation, Σ, of Mean for Offspring of 100 Mothers.

Serial Numb. in the Decade	Numb. of Siblings	Σ Directly Calc.	Σ Calc. by Formula	P. E. of the Mean
1	1	0.2111	0.1981	0.133
10	1	0.1938	0.1981	0.133
5. 6	2	0,1716	0.1704	0.115
1, 10	2	0.1757	0.1704	0.115
1. 6. 10	3	0.1627	0.1601	0.108
1, 2, 9, 10	4	0.1606	0.1547	0.104
4. 5. 6, 7, 8	5	0.1457	0.1514	0.102
2. 3. 5, 6, 8, 9	6	0.1502	0.1491	0.101
—	7	—	0.1475	0.100
2. 3. 4, ... 9	8	0.1446	0.1462	0.099
—	9	—	0.1453	0.098
1, 2, 3 10	10	0.1445	0.1445	0.097

For 10 siblings the theoretical value of Σ agrees exactly with the directly calculated, because the formula for r_f has been calculated from that value; for the same reason the agreement between the two values of Σ is especially good for great numbers of siblings, it is however on the whole satisfactory. It is seen that by taking two siblings from each mother, instead of one, the probable error is reduced by abt. 14 %. To diminish it further

by 14 % five or six embryos have to be added to each group of siblings.

VI. Personal and Generative Variation.

Dr. Johs. Schmidt found in his experiments with diallel crossings with trout[1]) the basis for the definition of what he has named generative values. The experiments consisted in this that each of a small number of females were crossed with each of a small number of males, after which the offspring were developed under equal environmental conditions. When it had reached a sufficient size a great number of each group of siblings were examined. In calculating the mean value for the examined character, the number of vertebrae, it appeared that the difference between the means for two groups of siblings ax and ay with the same mother a and with fathers x and y was equal to the difference between the means for the groups bx and by and therefore independent of the value of the mother.

It was hence possible to interpret the mean value for a group of siblings as a sum of two elements, one arising from the father and the other from the mother, plus a constant depending on the environments[2]). It was possible, when an arbitrary value was fixed for the element of one of the parents and for the constant depending on the environments, to calculate the rest of the elements of the parents from the means for the groups of offspring. Supposing that the offspring was equally influenced by the father and the mother Dr. Schmidt defined double the element received from the parent as the generative value of the father or the mother. It will be seen that the calculated generative values contain an unknown term, depending on the environments, but this term occurs with the same sign in parents of the same sex. Fully known are therefore differences between generative values of fathers and between generative values of mothers.

By calculating generative values it is endeavoured to characterize the individuals by the mean values of their possibilities

[1]) Comptes-rendus des Travaux du Laboratoire Carlsberg, Vol. **14**, Nr. 6. 1919, and "Diallel Crossings with Trout" in Journ. of Genet., Vol. IX. Nr. 1, 1919.

[2]) See Johs. Schmidt: Experimental Investigations with *Lebistes Reticulatus* (Peters) Regan (Journ. of Genet. Vol. VIII, Nr. 3, 1919).

as parents. Since whole siblings developed in the same environment occur with different personal values, it is, as long as the experiment is carried out with a small number of both parents, necessary to examine a great number of siblings to arrive at means from which the generative values can be accurately evaluated.

A comparison between the personal and the calculated generative value of a parent shows that they certainly resemble each other somewhat, but that they cannot by far be made to coincide by suitable choice of the unknown constant of the experiment. The deviations formed, which involve that two parents with equal generative values as a rule have different personal values, can be imagined partly to arise from different environments in the period of development, when the individuals could be influenced by environment, but it can also be imagined that whole siblings, developed in the same environments, with equal generative values have different personal values.

When the development of the embryos as in *Zoarces* has taken place under natural conditions, the environmental conditions can hardly be expected to have been approximately as uniform for the various groups of siblings as in the experiments with trout, which took place in the laboratory. It is more reasonable to assume that the means for various groups of offspring differ by unequal values from the means of the parental, generative values. With this alteration in the assumptions the hypothesis of generative values will be applied to the statistical results arrived at here on the assumption that offspring of the same mother are whole siblings.

Let the personal, observed value in a mother be x, while the supposed generative value is x_g. The deviation ε between these values is then supposed to be left to chance; in other words ε is independent of x_g, so that the correlation between ε and x_g is zero when a great number of mothers are considered. Denoting the standard deviations by σ_g and σ_ε, we therefore have

$$x = x_g + \varepsilon$$

and

$$\sigma_1^2 = \sigma_g^2 + \sigma_\varepsilon^2 \quad \text{(VI)}$$

For the fathers we have in a similar way

$$z = z_g + \varepsilon.$$

Our hypothesis is then, that for a great group of siblings with the parental values x and z, the mean is:

$$\bar{y} = \frac{1}{2}(x_g + z_g) + \delta,$$

where δ is independent of x_g and z_g, i. e. ruled by chance.

From the statistical investigations we know the standard deviation, σ_1, of x, the standard deviation, σ_∞, of $\bar{y}$, which is $\sigma_2 \sqrt{r_f}$, and the product moment of x and $\bar{y}$, which is $r_p \sigma_1 \sigma_2$. As it will be seen in the following, this gives three equations for determining σ_g, σ_ε and σ_δ, so that it is possible with these assumptions to separate from the variation found a generative variation.

Supposing that the standard deviation for paternal generative values is σ_g, as for the maternal, the standard deviation for $\bar{y}$ is is equal to $\frac{1}{2}\sigma_g^2 + \sigma_\delta^2$, so that:

$$\sigma_2 \sqrt{r_f} = \frac{1}{2}\sigma_g^2 + \sigma_\delta^2 \quad \ldots\ldots\ldots\ldots\ldots \text{(VII)}$$

For the product moment of x and y, referred to the respective means, is found $\frac{1}{2}\sigma_g^2$, the x_g, z_g, ε and δ having mutually according to the suppositions the correlations zero. We therefore have:

$$r_p \sigma_1 \sigma_2 = \frac{1}{2}\sigma_g^2 \quad \ldots\ldots\ldots\ldots\ldots \text{(VIII)}$$

or

$$\sigma_g = \sqrt{2 \sigma_1 \sigma_2 r_p},$$

by means of which are found from (VI) and (VII):

$$\sigma_\varepsilon = \sqrt{\sigma_1 (\sigma_1 - 2 r_p \sigma_2)}$$

and

$$\sigma_\delta = \sqrt{\sigma_2 (r_f \sigma_2 - r_p \sigma_1)}.$$

With the values reached in the previous sections for σ_1, σ_2, r_p, and r_f the numerical of values σ_g, σ_ε, and σ_δ are those given in the following table.

The probable errors given in the table are calculated by comparing the results for σ_g, σ_ε and σ_δ obtained for different years. For pigment spots in two out of five years σ_δ^2, is found to be negative and in the other three years positive, so that σ_δ can be considered equal to zero. For Vert. and Pd σ_δ however

Table 10.

	Vert.	Pd	Pigm. D_1
σ_1	1.962	0.609	1.539
σ_2	1.992	0.593	1.562
σ_g	1.764 ± 0.034	0.435 ± 0.012	1.327 ± 0.014
σ_ε	0.858 ± 0.041	0.426 ± 0.021	0.780 ± 0.010
σ_δ	0.555 ± 0.054	0.172 ± 0.014	imagin.

differs perceptibly from zero, which must be interpreted as denoting that there are factors, besides the hereditary, which vary from one group of siblings to another of the same age-group. Besides the differences in physical conditions which depend on the locality it may for instance be imagined that a difference in time for the fertilization would cause that the embryos at that time of their development when the characters may be influenced by temperature were subjected to different environmental conditions, even when they live in the same waters.

According to our hypothesis the st. d. found for mothers is a resultant of the st. d. of the generative values and of the random, i. e. of the generative values independent variations. For Pd these two standard deviations are of about equal size, while for Vert. and Pigm. the st. d. of the random deviations are only half as great as the st. d. of the generative values.

The deviation ε can be imagined to originate from various sources, which each of them tend to vary the environmental conditions at the time when these may influence the characters. There is in the first place difference of age, from which follows usually different environmental conditions during the development. Let the term of ε which originates from the different age be denoted by β, it ought then to be possible approximately to find σ_β as the st. d. of the annual means for embryos,

for σ_β^2 is found for Vert. Pd. and Pigm.

0.0027 0.0234 0.0225.

In the second place, as we have seen, even different groups of offspring of the same age-group must for Vert. and Pd. be supposed to be influenced by different environments, and the term δ must therefore also be contained in ε. To this comes that the personal values are discrete numbers so that the "rounding

off' has added an error of approximation to ε, by which $\sigma_\varepsilon{}^2$ has increased by $\frac{1}{12}$.

Putting now

$\varepsilon = \beta + \delta + v +$ error of approximation + constant,

we find for Vert. Pd. and Pigm.
the following values for σ_v: 0.58, 0.21 and 0.70.

A random deviation v thus still remains, which has to be explained either by differences occurring even between siblings with the same generative values, or by errors of observations, which on the whole must cause an increase of σ_ε. We may investigate how great the st. d. of the observations had to be to explain the deviations above. For this we required that $\sigma_\varepsilon{}^2$ should be diminished by $\sigma_v{}^2$.

We have found

$$\sigma_\varepsilon{}^2 = \sigma_1{}^2 - 2\, r_p\, \sigma_1\, \sigma_2,$$

where the last term, which is twice the product moment for x and y, is uninfluenced by errors of observations. From this it follows that the st. d. of the errors of observations had to be just σ_v, if the deviation v should be explained in this way, but for that the values found above for σ_v are, at any rate for pigment spots and vertebrae, too great. The assumption is therefore forced upon us that siblings with the same generative values may have different personal values.

VII. Summary.

This investigation concerns *Zoarces viviparus* only from Station 31 Nakkehage in Ise Fjord (in the north of the Danish island Sealand) and deals with the characters, number of vertebrae, number of pectoral fin rays, and number of pigment spots in the part of the dorsal fin consisting of soft rays. It is shown that these characters have the same mean values in males and females. The material for the investigation of inheritance consists of six samples of gravid females collected in the late autumn of the years 1914—1919. From each female has been examined 10 embryos and the total number of examined mothers are for vertebrae 857, for pectoral fin rays about the same number, and for pigment spots 488.

Section III contains the deduction of a method for calculation of fraternal correlation by means of the standard deviation of means of siblings. The method is usable when the number of siblings is the same for all families, and it makes the construction of fraternal correlation tables unnecessary.

In Section IV it is shown that the means for number of vertebrae for embryos as well as for mothers are essentially constant for the six samples, while the number of pigment spots and especially the number of pectoral fin rays in some cases vary perceptibly from one sample to another. Examples are given of regressions curves of offspring on mothers, which appear to be linear, and it is shown, also by examples, that the three characters follow the normal law of frequency.

For maternal correlation are found without Sheppard's correction for discontinuity:

Vert. 0.398, Pd. 0.262 and Pigm. 0,366,

and with Sheppard's correction:

Vert. 0.407, Pd. 0.329 and Pigm. 0.379.

These values are smaller than the parental correlation coefficients, found from adequate data, concerning physical characters in man, horse and dog. The resemble however the correlation coefficients found by R. C. Punnett from a small material for

countable characters in *Spinax niger*, being yet on the whole somewhat greater than these.

For fraternal correlation are found without Sheppard's correction:

Vert. 0.470, Pd. 0.353 and Pigm. 0.350,

and with Sheppard's correction:

Vert. 0.480, Pd. 0.420 and Pigm. 0.362.

These values are also smaller than those which are most frequently found from as extensive and good investigations as the present. Also these values resemble Punnett's results from *Spinax niger*, although they are, as were the parental correlation coefficients, somewhat greater than Punnett's values.

It is not possible by statistics to decide, whether the fraternal correlation concerns whole siblings. But a comparison with investigations which deal with correlation between both whole and half siblings as well as with parental correlation tells, in so much as similar numerical results generally can be expected, in favour of the supposition that we have to do with whole siblings.

In Section V various greater individual offspring samples are analysed with regard to number of vertebrae, and it is shown that a smaller number of such samples is not fit for calculation of fraternal correlation. Moreover, tables are given to show the accuracy obtained by increasing the number of siblings from one to ten.

In Section VI the consequences of Dr. Johs. Schmidt's hypothesis on generative values are followed up by aid of the present statistical results assuming that all offspring of the same mother are whole siblings. It appears that we are forced to assume that groups of siblings from the same age-group as to number of vertebrae and pectoral fin rays are influenced by different environmental conditions, and moreover that siblings with the same generative values may have different personal values.

VIII. Tables.

A. Correlation Tables.

Table 11. — Vert. Maternal Correlation 1914.

Offspring \ Mothers	118	117	116	115	114	113	112	111	110	109	108	Total
122	»	1	»	»	»	»	»	»	»	»	»	1
121	»	»	»	»	»	»	»	»	»	»	»	»
120	»	1	»	1	»	»	»	»	»	»	»	2
119	1	5	»	»	1	»	»	»	»	»	»	7
118	2	2	»	»	5	1	2	»	»	»	»	12
117	2	1	5	14	10	9	1	1	»	»	»	43
116	8	5	15	19	18	19	16	1	»	»	»	101
115	3	5	20	37	38	42	26	4	1	»	»	176
114	4	15	31	44	51	66	30	9	3	1	»	254
113	»	11	23	36	55	87	55	9	11	»	»	287
112	»	2	19	20	47	64	39	12	11	6	2	222
111	»	2	16	15	22	41	18	10	28	2	1	155
110	»	»	6	3	15	22	9	2	18	»	2	77
109	»	»	4	1	7	7	2	2	5	»	1	29
108	»	»	»	»	1	1	1	»	2	1	2	8
107	»	»	1	»	»	1	1	»	1	»	1	5
106	»		»	»	»	»	»	»	»	»	1	1
Total...	20	50	140	190	270	360	200	50	80	10	10	1380

Table 12. — Vert. Maternal Correlation 1915.

Offspring \ Mothers	119	118	117	116	115	114	113	112	111	110	109	108	107	Total
120	1	»	1	»	»	»	»	»	»	»	»	»	»	2
119		»	1	1	1	1	2	»	»	»	»	»	»	6
118	5	»	7	7	5	2	»	3	»	»	»	»	»	29
117	3	»	11	17	8	3	5	4	2	1	»	»	»	54
116	5	»	13	20	25	21	14	19	11	2	»	»	»	130
115	5	»	17	23	42	33	32	36	25	3	»	»	»	216
114	1	»	19	19	37	48	65	81	32	1	1	»	»	304
113	»	»	13	11	33	60	72	93	69	6	2	»	1	360
112	»	»	3	8	29	44	67	57	60	8	3	»	»	279
111	»	»	2	2	6	14	37	47	36	11	2	»	1	158
110	»	»	3	2	2	10	15	21	22	11	2	»	2	90
109	»	»	»	»	2	3	6	9	8	6	»	»	3	37
108	»	»	»	»	»	1	3	»	4	»	»	»	2	10
107	»	»	»	»	»	»	2	»	»	1	»	»	1	4
106	»	»	»	»	»	»	»	»	1	»	»	»	»	1
Total	20	»	90	110	190	240	320	370	270	50	10	»	10	1680

Table 13. — Vert. Maternal Correlation 1916.

Offspring	Mothers 118	117	116	115	114	113	112	111	110	109	108	107	Total
120	»	1	»	»	»	»	»	»	»	»	»	»	1
119	»	2	»	»	»	»	»	»	»	»	»	»	2
118	»	2	»	»	»	3	»	»	»	»	»	»	5
117	»	7	10	4	3	5	2	»	»	»		»	31
116	1	15	17	7	21	16	11	7	»	»	»	»	95
115	1	18	26	18	41	31	22	6	4	»	»	»	167
114	4	9	35	19	42	54	33	31	5	1	»	»	233
113	1	9	35	30	42	53	38	39	11	4	»	»	262
112	2	6	17	16	34	48	27	30	10	6	»	»	196
111	1	1	5	5	10	28	24	24	11	6	»	1	116
110	»	»	3	1	6	14	24	17	6	8	»	4	83
109	»	»	»	»	1	6	8	4	3	3	»	2	27
108	»	»	2	»	»	2	1	1	»	1	»	2	9
107	»	»	»	»	»	»	»	1	»	1	»	»	2
106	»	»	»	»	»		»	»	»	»	»	1	1
Total...	10	70	150	100	200	260	190	160	50	30	»	10	1230

Table 14. — Vert. Maternal Correlation 1917.

Offspring	Mothers 118	117	116	115	114	113	112	111	110	109	108	Total
121	1	»	»	»	»	»	»	»	»	»	»	1
120	1	»	»	»	»	»	»	»	»	»	»	1
119	»	»	1	»	3	»	3	»	»	»	»	7
118	3	2	3	1	4	5	4	1	»	»	»	23
117	2	5	11	9	8	15	4	8	1	»	»	63
116	1	10	18	17	21	34	17	3	5	»	»	126
115	2	18	26	28	55	55	25	18	6	»	2	235
114	»	11	25	60	78	93	42	27	11	»	»	347
113	»	9	11	35	69	104	62	44	13	2	»	349
112	»	2	11	33	44	82	43	48	27	1	3	294
111	»	3	3	6	18	54	40	32	17	3	7	183
110	»	»	1	»	14	18	14	13	11	4	3	78
109	»	»	»	1	5	6	9	4	9	»	4	38
108	»	»	»	»	1	4	6	2	6	»	1	20
107	»	»	»	»	»	»	»	»	2	»	»	2
106	»	»	»	»	»	»	1	»	1	»	»	2
105	»	»	»	»	»	»	»	»	1	»	»	1
Total...	10	60	110	190	320	470	270	200	110	10	20	1770

Table 15. — Vert. Maternal Correlation 1918.

Offspring	Mothers 119	118	117	116	115	114	113	112	111	110	109	108	Total
118	»	3	»	1	2	8	5	»	»	»	»	»	19
117	3	1	1	2	13	20	6	2	3	»	»	»	51
116	4	6	6	10	35	30	18	14	9	1	»	»	133
115	2	6	5	14	48	45	43	19	14	2	2	»	200
114	1	»	7	19	64	68	51	43	27	9	»	»	289
113	»	4	1	8	45	69	51	61	34	29	8	3	313
112	»	»	»	2	24	55	39	34	40	24	6	5	229
111	»	»	»	3	25	23	27	19	36	25	11	4	173
110	»	»	»	1	5	12	9	7	11	22	8	8	83
109	»	»	»	»	8	»	»	1	4	3	3	5	24
108	»	»	»	»	1	»	1	»	»	4	1	4	11
107	»	»	»	»	»	»	»	»	1	1	1	1	4
106	»	»	»	»	»	»	»	»	1	»	»	»	1
Total...	10	20	20	60	270	330	250	200	180	120	40	30	1530

Table 16. — Vert. Maternal Correlation 1919.

Offspring	Mothers 117	116	115	114	113	112	111	110	109	108	Total
118	1	»	4	2	4	»	1	»	»	»	12
117	2	4	6	4	7	3	1	»	»	»	27
116	»	12	19	9	10	4	7	1	»	»	62
115	1	24	17	23	19	17	10	4	»	»	115
114	7	17	36	42	33	15	30	7	2	»	189
113	5	13	33	35	41	36	38	10	2	1	214
112	3	7	21	19	45	22	47	10	1	2	177
111	1	2	12	11	17	12	36	11	2	4	108
110	»	1	2	5	8	7	15	9	2	3	52
109	»	»	»	»	4	3	4	5	1	»	17
108	»	»	»	»	2	1	1	3	»	»	7
Total...	20	80	150	150	190	120	190	60	10	10	980

Table 17. — Vert. Maternal Correlation 1914—1919.

Offspring	Mothers 119	118	117	116	115	114	113	112	111	110	109	108	107	Total
122	»	»	1	»	»	»	»	»	»	»	»	»	»	1
121	»	1	»	»	»	»	»	»	»	»	»	»	»	1
120	1	1	3	»	1	»	»	»	»	»	»	»	»	6
119	»	1	8	2	1	5	2	3	»	»	»	»	»	22
118	5	8	14	11	12	21	18	9	2	»	»	»	»	100
117	6	5	27	49	54	48	47	16	15	2	»	»	»	269
116	9	16	49	92	122	120	111	81	38	9	»	»	»	647
115	7	12	64	133	190	235	222	145	77	20	2	2	»	1109
114	2	8	68	146	260	329	362	244	156	36	5	»	»	1616
113	»	5	48	101	212	330	408	345	233	80	18	4	1	1785
112	»	2	16	64	143	243	345	222	237	90	23	12	»	1397
111	»	1	9	31	69	98	204	160	174	103	26	16	2	893
110	»	»	3	14	13	62	86	82	80	77	24	16	6	463
109	»	»	»	4	12	16	29	32	26	31	7	10	5	172
108	»	»	»	2	1	3	13	9	8	15	3	7	4	65
107	»	»	»	1	»	»	3	1	2	5	2	2	1	17
106	»	»	»	»	»	»	»	1	2	1	»	1	1	6
105	»	»	»	»	»	»	»	»	»	1	»	»	»	1
Total...	30	60	310	650	1090	1510	1850	1350	1050	470	110	70	20	8570

Pd. Maternal Correlation.

Table 18. 1914.

		Mothers				Total
		21	20	19	18	
Offspring	21	»	4	3	»	7
	20	4	271	176	1	452
	19	6	334	399	25	764
	18	»	21	62	14	97
Total...		10	630	640	40	1320

Table 19. 1915.

		Mothers				Total
		21	20	19	18	
Offspring	21	»	24	15	»	39
	20	6	278	296	8	588
	19	4	252	675	85	1016
	18	»	6	64	26	96
	17	»	»	»	1	1
Total...		10	560	1050	120	1740

Table 20. 1916.

		Mothers				Total
		21	20	19	18	
Offspring	21	»	1	»	»	1
	20	5	106	99	8	218
	19	5	265	481	64	815
	18	»	27	138	18	183
	17	»	1	2	»	3
Total...		10	400	720	90	1220

Table 21. 1917.

		Mothers				Total
		21	20	19	18	
Offspring	21	3	6	1	»	10
	20	13	172	173	9	367
	19	12	310	768	87	1177
	18	2	22	147	34	205
	17	»	»	1	»	1
Total...		30	510	1090	130	1760

Table 22. 1918.

		Mothers				Total
		21	20	19	18	
Offspring	21	1	8	4	»	13
	20	13	222	269	16	520
	19	5	213	626	90	934
	18	1	7	61	24	93
Total...		20	450	960	130	1560

Table 23. 1919.

		Mothers			Total
		20	19	18	
Offspring	21	11	12	1	24
	20	156	222	41	419
	19	152	252	107	511
	18	1	14	11	26
Total...		320	500	160	980

Ps. Maternal Correlation.

Table 24. 1914.

		Mothers				Total
		21	20	19	18	
Offspring	21	»	7	1	»	8
	20	4	288	181	1	474
	19	6	330	370	30	736
	18	»	15	47	9	71
	17	«	»	1	»	1
Total...		10	640	600	40	1290

Table 25. 1916.

		Mothers				Total
		21	20	19	18	
Offspring	21	»	»	»	»	»
	20	4	102	132	2	240
	19	6	253	500	54	813
	18	»	25	115	14	154
	17	»	»	3	»	3
Total...		10	380	750	70	1210

Table 26. 1917.

		Mothers				Total
		21	20	19	18	
Offspring	21	3	4	3	»	10
	20	9	221	189	2	421
	19	7	326	769	47	1149
	18	1	19	128	21	169
	17	»	»	1	»	1
Total...		20	570	1090	70	1750

Table 27. 1918.

		Mothers				Total
		21	20	19	18	
Offspring	21	2	10	5	»	17
	20	12	262	297	25	596
	19	6	213	552	108	879
	18	»	5	46	17	68
Total...		20	490	900	150	1560

Table 28. 1919.

		Mothers				Total
		21	20	19	18	
Offspring	21	1	8	9	1	19
	20	6	185	227	29	447
	19	3	145	274	74	496
	18	»	2	10	6	18
Total...		10	340	520	110	980

Table 29. Pigm. D_1. Maternal Correlation 1915.

Offspring	Mothers 20	19	18	17	16	15	14	13	12	11	10	Total
19	1	»	»	»	1	»	»	1	»	»	»	3
18	4	»	»	»	»	»	1	1	»	»	»	6
17	2	»	»	»	2	5	2	3	»	1	»	15
16	2	»	»	»	4	15	12	10	3	2	»	48
15	1	»	»	»	4	40	43	12	12	7	»	119
14	»	»	»	»	7	58	62	44	34	3	1	209
13	»	»	»	»	2	49	53	42	36	18	3	203
12	»	»	»	»	»	20	23	22	25	7	1	98
11	»	»	»	»	»	13	4	5	10	8	1	41
10	»	»	»	»	»	»	»	»	»	3	3	6
9	»	»	»	»	»	»	»	»	»	1	1	2
Total...	10	»	»	»	20	200	200	140	120	50	10	750

Table 30. Pigm. D_1. Maternal Correlation 1916.

Offspring	Mothers 17	16	15	14	13	12	11	Total
19	»	»	»	1	1	»	»	2
18	»	1	3	5	»	»	1	10
17	»	5	4	8	5	»	1	23
16	1	16	16	23	19	1	2	78
15	4	18	40	41	18	15	6	142
14	3	19	46	54	39	16	15	192
13	2	8	38	54	60	33	24	219
12	»	3	8	38	38	31	18	136
11	»	»	5	6	13	19	10	53
10	»	»	»	»	7	4	»	11
9	»	»	»	»	»	1	3	4
Total...	10	70	160	230	200	120	80	870

Table 31. Pigm. D_1. Maternal Correlation 1917.

		Mothers									Total
		18	17	16	15	14	13	12	11	10	
Offspring	19	»	»	2	»	»	»	»	»	»	2
	18	2	»	4	»	4	1	»	»	»	11
	17	1	»	11	4	7	5	1	»	»	29
	16	4	5	24	10	17	15	5	3	1	84
	15	7	11	13	33	62	43	17	3	1	190
	14	2	14	23	66	114	63	32	18	1	333
	13	1	7	9	54	89	56	44	32	4	296
	12	3	3	2	25	46	50	40	23	3	195
	11	»	»	2	8	21	21	16	15	3	86
	10	»	»	»	»	7	4	5	11	6	33
	9	»	»	»	»	3	2	»	5	1	11
Total...		20	40	90	200	370	260	160	110	20	1270

Table 32. Pigm. D_1. Maternal Correlation 1918.

		Mothers									Total
		19	18	17	16	15	14	13	12	11	10	
Offspring	18	»	»	»	»	1	»	»	»	»	»	1
	17	1	»	2	5	4	5	4	»	»	»	21
	16	3	»	8	7	15	15	9	1	»	»	58
	15	2	»	16	12	40	37	21	9	1	1	139
	14	2	»	14	11	55	110	38	24	12	3	269
	13	2	»	14	11	68	105	63	48	17	1	329
	12	»	»	4	2	27	76	29	37	18	5	198
	11	»	»	2	1	9	23	5	22	11	5	78
	10	»	»	»	1	1	9	1	7	8	5	32
	9	»	»	»	»	»	»	»	1	3	»	4
	8	»	»	»	»	»	»	»	1	»	»	1
Total...		10	»	60	50	220	380	170	150	70	20	1130

Table 33. Pigm. D_1. Maternal Correlation 1919.

Offspring	Mothers 18	17	16	15	14	13	12	11	Total
20	»	»	»	»	1	»	»	»	1
19	1	»	»	»	»	»	»	»	1
18	2	»	»	1	2	»	»	»	5
17	2	1	3	4	3	»	»	»	13
16	6	7	8	16	16	3	5	»	61
15	1	4	23	30	39	21	8	1	127
14	1	1	21	44	62	36	43	3	211
13	2	5	13	45	54	58	42	5	224
12	2	1	9	14	30	43	27	7	133
11	2	1	2	5	12	23	5	8	58
10	1	»	1	1	1	8	»	4	16
9	»	»	»	»	»	6	»	1	7
8	»	»	»	»	»	2	»	1	3
Total...	20	20	80	160	220	200	130	30	860

Table 34. Vert. Means of Decades and their Squared Standard Deviations (σ_d^2).

Means of Decades	1914 Freq.	1915 Freq.	1916 Freq.	1917 Freq.	1918 Freq.	1919 Freq.	1914—1919 Freq.
119.25 — 118.75	1	»	»	»	»	»	1
118.75 — 118.25	»	»	»	»	»	»	»
118.25 — 117.75	»	»	»	»	»	»	»
117.75 — 117.25	»	»	»	1	»	»	1
117.25 — 116.75	1	3	»	1	»	»	5
116.75 — 116.25	»	2	2	3	1	1	9
116.25 — 115.75	2	6	2	3	5	2	20
115.75 — 115.25	5	4	4	6	6	1	26
115.25 — 114.75	8	11	4	10	12	9	54
114.75 — 114.25	12	11	16	18	17	8	82
114.25 — 113.75	20	18	14	23	18	10	103
113.75 — 113.25	20	30	19	22	17	12	120
113.25 — 112.75	17	25	23	26	22	15	128
112.75 — 112.25	20	18	8	23	16	15	100
112.25 — 111.75	8	19	13	13	16	13	82
111.75 — 111.25	9	9	3	13	10	7	51
111.25 — 110.75	11	6	5	6	6	1	35
110.75 — 110.25	1	2	4	3	4	4	18
110.25 — 109.75	2	1	5	4	1	»	13
109.75 — 109.25	1	2	»	2	1	»	6
109.25 — 108.75	»	1	1	»	1	»	3
Total...	138	168	123	177	153	98	857
Squared St. D. of Means of Decades (σ_d^2)	2.10755	2.13939	2.12035	2.17137	2.08742	1.63941	2.07380

Table 35. Pd. Means of Decades and their Squared Standard Deviations ($\sigma_d{}^2$).

Means of Decades	1914 Freq.	1915 Freq.	1916 Freq.	1917 Freq.	1918 Freq.	1919 Freq.
20.6	»	2	»	»	»	»
20.5	»	1	»	»	»	»
20.4	»	1	»	»	»	»
20.3	»	2	»	2	»	»
20.2	»	1	»	»	»	3
20.1	1	2	»	»	3	3
20.0	2	1	»	3	3	4
19.9	4	7	1	2	6	6
19.8	8	12	4	3	10	5
19.7	9	12	2	6	7	7
19.6	5	6	6	5	11	8
19.5	12	13	5	9	10	10
19.4	16	15	4	11	11	9
19.3	22	19	11	8	23	14
19.2	8	20	8	15	13	8
19.1	12	10	19	33	16	8
19.0	5	17	12	21	19	8
18.9	9	19	16	20	5	1
18.8	7	3	9	14	8	2
18.7	6	4	6	5	6	2
18.6	3	3	5	6	1	»
18.5	1	2	5	6	3	»
18.4	1	»	4	3	1	»
18.3	»	1	1	3	»	»
18.2	»	1	»	»	»	»
18.1	1	»	1	»	»	»
18.0	»	»	1	1	»	»
17.9	»	»	2	»	»	»
Total...	132	174	122	176	156	98
Squared St. D. of Means of Decades ($\sigma_d{}^2$)	0.138294	0.180795	0.156485	0.144995	0.136638	0.125969

Table 36. Ps. Means of Decades and their Squared Standard Deviations (σ_d^2).

Means of Decades	1914 Freq.	1916 Freq.	1917 Freq.	1918 Freq.	1919 Freq.
20.3	»	»	1	1	1
20.2	»	»	»	2	1
20.1	2	»	1	1	5
20.0	4	»	3	7	5
19.9	4	2	3	6	2
19.8	8	5	9	9	6
19.7	12	2	5	10	7
19.6	5	5	6	9	8
19.5	16	3	7	17	14
19.4	12	9	14	14	14
19.3	12	5	13	18	7
19.2	10	18	13	16	12
19.1	13	13	27	9	11
19.0	8	14	23	15	2
18.9	6	16	17	11	1
18.8	10	9	12	6	»
18.7	4	8	6	3	1
18.6	1	2	4	1	1
18.5	1	3	6	»	»
18.4	»	2	3	1	»
18.3	»	1	2	»	»
18.2	»	2	»	»	»
18.1	1	»	»	»	»
18.0	»	1	»	»	»
17.9	»	1	»	»	»
Total...	129	121	175	156	98
Squared St. D. of Means of Decades (σ_d^2).....	0.143800	0.145215	0.147853	0.136369	0.111388

Table 37. Pigm. D_1. Means of Decades and their Squared Standard Deviations ($\sigma_d{}^2$).

Means of Decades	1915 Freq.	1916 Freq.	1917 Freq.	1918 Freq.	1919 Freq.
17.35 — 17.05	1	»	1	»	»
17.05 — 16.75	»	»	»	»	1
16.75 — 16.45	»	1	»	»	»
16.45 — 16.15	»	»	1	»	1
16.15 — 15.85	1	1	»	»	»
15.85 — 15.55	»	3	»	»	1
15.55 — 15.25	1	2	5	1	»
15.25 — 14.95	3	4	2	4	5
14.95 — 14.65	2	1	5	4	3
14.65 — 14.35	2	7	14	5	2
14.35 — 14.05	13	12	8	10	6
14.05 — 13.75	10	8	21	10	7
13.75 — 13.45	10	9	15	13	16
13.45 — 13.15	10	15	18	15	15
13.15 — 12.85	13	7	5	18	7
12.85 — 12.55	3	5	8	14	10
12.55 — 12.25	2	5	4	6	4
12.25 — 11.95	1	4	4	4	2
11.95 — 11.65	1	»	7	3	3
11.65 — 11.35	2	2	4	3	»
11.35 — 11.05	»	1	4	2	»
11.05 — 10.75	»	»	1	1	2
10.75 — 10.45	1	»	»	»	1
Total...	75	87	127	113	86
Squared St. D. of Means of Decades ($\sigma_d{}^2$)....	0.84300	1.07088	1.24047	0.83117	1.11173

Carlsberg Laboratory, 2. November 1920.

POSTSCRIPT.

After reading the danish version of this paper, which has been ready in print for a few weeks, Dr. Georg Duncker has sent the following note to the Laboratory:

Remark on the reciprocal relations between mothers and offspring in *Zoarces viviparus* L.

Let I be the number of vertebrae in mothers, II the same character in offspring, V a single observed numerical value thereof with the frequency f, A the arithmetic mean of all the 'V's, s their standard deviation, n the total numbers of weighted mothers and of offspring, then:

$$A_I = \frac{1}{n} \Sigma (V_I) \qquad A_{II} = \frac{1}{n} \Sigma (V_{II})$$

$$s_I = \sqrt{\frac{1}{n} \Sigma (V_I - A_I)^2} \qquad s_{II} = \sqrt{\frac{1}{n} \Sigma (V_{II} - A_{II})^2}$$

$$\beta_{r,o} = \frac{\frac{1}{n} \Sigma (V_I - A_I)^r}{s_I{}^r} \qquad \beta_{o,r} = \frac{\frac{1}{n} \Sigma (V_{II} - A_{II})^r}{s_{II}{}^r}.$$

Further, let A_I' be the arithmetic mean of the mothers of all embryos with a certain given number of vertebrae, s_I' their standard deviation, A_{II}' the arithmetic mean of the offspring of all mothers with a certain given number of vertebrae, s_{II}' their standard deviation, then:

$$s_I' = \sqrt{\frac{1}{f_{II}} \Sigma (V_I - A_I')^2} \qquad s_{II}' = \sqrt{\frac{1}{f_I} \Sigma (V_{II} - A_{II}')^2}$$

$$[s_I']^2 = \frac{\Sigma f_{II} \, s_I'^2}{n} \qquad [s_{II}']^2 = \frac{\Sigma f_I \, s_{II}'^2}{n}.$$

Finally, if

$$\beta_{\mu,\nu} = \frac{1}{n} \Sigma [(V_I - A_I)^\mu (V_{II} - A_{II})^\nu] : s_I{}^\mu \, s_{II}{}^\nu,$$

then $\beta_{1,1}$ is the well known coefficient of correlation.

Now Pearson (1905, p. 11)[1]) has shown, that if the correlation is linear:

$$\beta_{1,1}{}^2 = 1 - \frac{[s_I']^2}{s_I{}^2} = 1 - \frac{[s_{II}']^2}{s_{II}{}^2},$$

but if it is not:

$$\beta_{1,1}{}^2 < 1 - \frac{[s_I']^2}{s_I{}^2}$$
$$< 1 - \frac{[s_{II}']^2}{s_{II}{}^2},$$

both differences on the right hand side not necessarily being equal.

From the present data (p. 44, Table 17) I find:

$$1 - \frac{[s_I']^2}{s_I{}^2} = 0.16004.$$
$$1 - \frac{[s_{II}']_2}{s_{II}{}^2} = 0.16775.$$
$$\beta_{1,1}{}^2 = 0,15845.$$

The difference:

$$1 - \frac{[s_{II}']^2}{s_{II}{}^2} - \beta_{1,1}{}^2 = 0.00930$$

is nearly six times as large as the difference:

$$1 - \frac{[s_I']^2}{s_I{}^2} - \beta_{1,1}{}^2 = 0.00159.$$

The standard error of the former is given by Dr. K. Smith (p. 11) as:

$$\sigma_z = 0.0046.$$

This difference therefore is 2.02 times its standard error and the probability of its being significant:

$$\Phi(2.02) = 0.95626$$

or about 22 : 1.

If we assume the standard error of the second difference to be the same, we find accordingly:

[1]) K. Pearson: Mathematical contributions to the theory of evolution. — — XIV. On the general theory of skew correlation and non-linear regression. — Draper's Co. Res. Mem. Biometr. Ser. II. London 1905.

$$\Phi\,(0.345) = 0.2699$$

or a probability of 2.7 : 1 against its being significant.

In the first case the regression line cannot be straight, and since the straight regression line is cutting the observed one in three points, viz. at — 1.30, 0.16 and 1.86 of its absolute abscissa (see Fig. 2), the theoretical regression curve of offspring on mothers ought to be of the third order, or:

$$y_{II} = \gamma_0 + \gamma_1\ x_I + \gamma_2\ x_I^2 + \gamma_3\ x_I^3.$$

On the other hand, the observed regression line of mothers on offspring, apart from three and two points respectively at either extremity based upon observations of mothers of 15 embryos only altogether, is practically straight and therefore determined by the first order equation:

$$y_I = \beta_{1,1}\ x_{II}.$$

We thus find a distinct difference between the regression of offspring on mothers and that of mothers on offspring, which can be recognized only by applying Pearson's method of higher product-moments to the problems of heredity and correlation.

The numerical values of the constants A, s, β and γ are given below (Table I), so are the values A' (Table II) both observed and calculated by means of the equations (regression of mothers on offspring; see Fig. 1):

$$L:\ y_1 = 0.39806\ x$$
$$P_2:\ y_2 = 0.00512 + 0.39823\ x - 0.00512\ x^2$$

(regression of offspring on mothers; see Fig. 2):

$$L:\ y_1 = 0.39806\ x$$
$$P_3:\ y_3 = 0.01783 + 0.30401\ x - 0.01815\ x^2 + 0.03537\ x^3,$$

where, in the first case:

$$y = \frac{A_I' - A_I}{s_I}, \quad x = \frac{V_{II} - A_{II}}{s_{II}},$$

in the second:

$$y = \frac{A_{II}' - A_{II}}{s_{II}}, \quad x = \frac{V_I - A_I}{s_I}.$$

While there is no great difference between the results of the two first equations, the fourth gives a remarkably closer fit

Fig. 1.

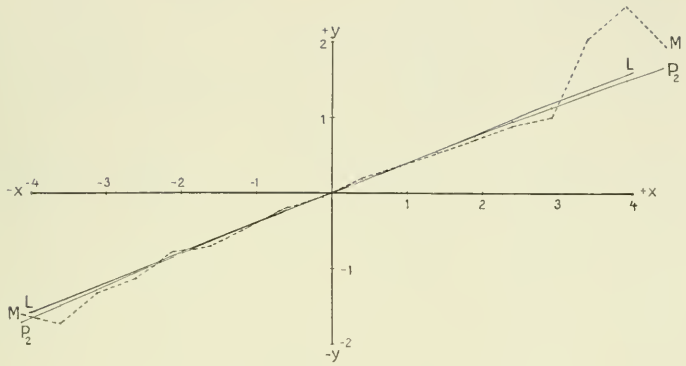
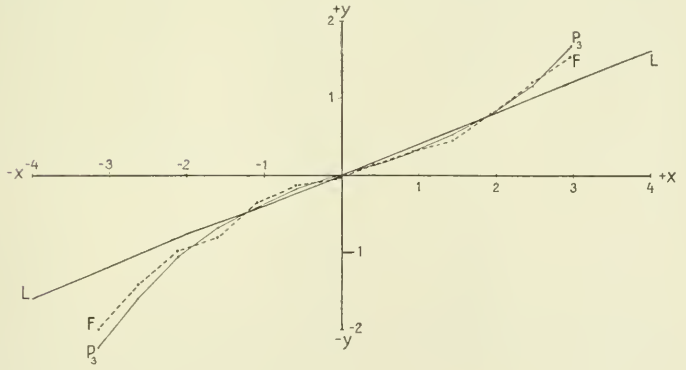


Fig. 2.



than the third, as will be seen at once from the mean square differences (Δ) between observations and calculations:

Fig. 1: $\Delta(L) = 0.03982$ $\Delta(P_2) = 0.03765$
$= 0.07821$ vertebrae $= 0.07385$ vertebrae.
Fig. 2: $\Delta(L) = 0.09638$ $\Delta(P_3) = 0.05075$
$= 0.19202$ vertebrae $= 0.10110$ vertebrae.

Hamburg, 28. January 1921.

GEORG DUNCKER.

Table I.

	1. Variation-constants of I. Mothers	1. Variation-constants of II. Offspring	2. Correlation	
A	113.18553	113.21062	β_{11}	0.39806
s	1.96156	1.99219	β_{21}	— 0.03946
β_3	0.00991	— 0.03342	β_{12}	— 0.02441
β_4	2.94842	3.17001	β_{31}	1.35246
β_5	— 0.21242	— 0.35007	β_{13}	1.19581
β_6	14.14533	17.63168		

	3. Regression-constants of Mothers on offspring P_2	Mothers on offspring P_3	Offspring on mothers P_2	Offspring on mothers P_3
γ_0	0.00512	0.00570	0.02228	0.01783
γ_1	0.39823	0.42607	0.39828	0.30401
γ_2	— 0.00512	— 0.00600	— 0.02228	— 0.01815
γ_3	»	— 0.00890	»	0.03537

Table II.

	A_I' of Mothers (Fig. 1)			A_{II}' of Offspring (Fig. 2)		
	Obs.	Calc.		Obs.	Calc.	
V	M	L	P_2	F	L	P_3
105	110.000	109.967	109.809	»	»	»
106	109.833	110.359	110.237	»	»	»
107	110.588	110.751	110.663	109.250	110.710	108.767
108	111.000	111.143	111.084	110.400	111.114	110.091
109	111.645	111.535	111.500	111.245	111.519	111.105
110	111.827	111.927	111.911	111.596	111.923	111.865
111	112.280	112.319	112.316	112.523	112.327	112.429
112	112.769	112.711	112.717	112.948	112.731	112.852
113	113.070	113.103	113.113	113.118	113.136	113.188
114	113.574	113.495	113.505	113.518	113.540	113.496
115	113.897	113.887	113.889	113.846	113.944	113.831
116	114.215	114.279	114.221	114.155	114.349	114.249
117	114.561	114.671	114.645	114.794	114.753	114.807
118	114.910	115.063	115.015	115.617	115.157	115.557
119	115.136	115.455	115.381	116.300	115.561	116.559
120	117.167	115.847	115.790	»	»	»
121	118.000	116.239	116.096	»	»	»
122	117.000	116.630	116.446	»	»	»
I		0.078	0.074		0.192	0.101

There is no doubt that for vertebrae a much better fit is obtained to the means of offspring for constant values of mothers by a cubic than by a linear regression curve. It is clearly brought forward by Dr. Duncker's figures and was to be expected after a glance at Fig. 1 of my treatise.

As to the significance of the deviation from linear regression I am not convinced from the present observations, and less so now, when I have examined the samples more closely, than when I had only considered the compound of 6 samples.

For insisting upon a deviation of twice the standard deviation being significant one must be rather critical as to the purity of the material in hand. It now appears that while for the five samples the mean values agree pretty well for mothers as well as for offspring, the mean of mothers in the 1914-sample deviates by more than 3 times the probable error of the deviation from some of the other means. For the conclusions otherwise drawn from the results this is of no consequence, but if the results shall be pressed to this point one has to be more careful.

To obtain a more homogeneous compound of observations I have therefore excluded the 1914-sample. For the compound of samples 1915—1919 I find with the notations from Section IV A:

$\eta^2 = 0.17400$, $r^2 = 0.16722$, and the crucial deviation ζ equal to 0.00677 with st. d. 0.00431. We have thus arrived at a deviation which is 1.57 times the st. d.

It therefore appears to me that while the cubic gives an extremely good description of the results of these observations, it is by no means proved that this deviation from linear form is of real significance.

The Laboratory is in position of another material for investigation of inheritance of number of vertebrae in *Zoarces*. It will be interesting to learn whether it discloses signs of cubic regression.

Carlsberg Laboratory, 11 Febr. 1921.

KIRSTINE SMITH.

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COMPTES-RENDUS

DES TRAVAUX

DU

LABORATOIRE CARLSBERG

14ME VOLUME.

No. 12

COPENHAGUE

EN COMMISSION CHEZ H. HAGERUP

IMPRIMERIE DE THIELE

1921

Prix: 1 Kr. 20 Øre.

ON A PARTIAL SEX-LINKED INHERITANCE OF EYE-COLOUR IN MAN.

BY

Ö. WINGE.

I. Introduction.

Hurst's and Davenport's researches into the conditions of inheritance of eye-colour in man led to the conclusion that blue eye-colour (simplex) is recessive towards brown (duplex), and that inheritance takes place under the Mendelian scheme for monofactorial segregation. In case both parents have blue eyes, i. e. lack brown pigment on the front of the iris all their children will have blue eyes, while a brown-eyed parent and a blue-eyed one or even two brown-eyed parents — always provided none of them is homozygously brown-eyed — may have children with both brown and blue eyes.

H. Bryn (1920) objects to this supposition; though in the main arriving at similar results, he is absolutely certain to have shown exceptions in his Norwegian material. Bryn has examined 30 marriages in all between persons with blue eyes, but he came to the conclusion that only in 26 out of these 30 marriages all the children were blue-eyed (viz. 72 children in all), whereas there were 10 children with brown eyes and 17 with blue eyes in the remaining 4 marriages. However, Bryn shows that among the grandparents of the said 10 brown-eyed children there was in all 4 cases one or two with brown eyes, and he gathers herefrom, that "If both parents have blue eyes while some of the grandparents have eyes with brownish pigment, some of the children (ca. 10 %) will also have eyes with brownish pigment, while the rest will have blue ones."

The formulation of this reputed lawfulness is, it is true, not very well-chosen, for in case a general mendling brown factor may be recessive it may, of course, be found cryptome-

rically in more than one generation; thus not even the grandparents need to have had brown eyes but possibly earlier ancestors. The validity of the statement "10 %" is also very doubtful. An investigation of the grandparents of the children of the remaining 26 families would surely have led to the demonstration of grandparents, now with brown eyes, now all with blue eyes. The 10 % has, as will be easily seen, nothing to do with the number per cent of brown eyes in such children with blue-eyed parents as have one or more brown-eyed grandparents.

This, however, is only a wrong statistical formulation which has nothing to do with the reality, Bryn thinks to have shown, viz. that parents with blue eyes may have children with brown eyes. I shall later revert to this question.

On the following pages I shall try briefly to account for some researches on the inheritance of eyecolour made of late years, and the results to which these researches have led me.

II. Provisional treatment of my material.

In 1918 I sent out a few hundred questionnaires to friends and acquaintances as also to members of two associations of natural history in Denmark. The questionnaires contained questions as to the eye-colour of the person himself, as also the eye-colour of his father, the brothers and sisters of his father and of the parents of his father. The questionnaires contained similar questions as to the eye-colour of his mother's family and finally as to the eye-colour of his wife and her family and that of his own children. For the guidance of the persons in question the following observations were printed on the questionnaires:

"The eye-colour is e. g. indicated as: light blue, middle blue, iron-grey (bluish grey), dark blue, yellowish brown, bluish brown, brownish green, dark brown, blackish brown, jetty black. In case the shade cannot be stated with absolute certainty, only "blue" or "brown" is stated. — Please state the age of persons below 16. — In many cases the person concerned will not be able to answer all questions with certainty. Consequently it must be pointed out that only correct answers are of any value. In case of uncertainty, please do not answer the question."

As might be expected several questionnaires were not answered, whereas many others were elaborated with great care. Where I had reason to suppose that certain statements were incorrect, I wrote to the person concerned (except in such cases where the address was unknown to me) asking for a renewed examination. This held good of all cases in which parents with blue eyes were stated to have one or more children with brown eyes.

A great many details as to the eye-colour of brothers and sisters were of course wasted, because the eye-colour of only one or none of the parents was known with certainty. After a judicious sorting out of a few questionnaires with doubtful details which, owing to my not knowing the domiciles of the persons in question, could not be further controlled, the remaining material was treated statistically and gave the following result:

Tabel 1.

Marriages	Number of Children			In all
	Blue	Brown	Greyish green bluish green	
Blue × blue	62 5	12	7	644
Blue × brown and conversely	317	322	9	648
Brown × brown.....	25	82	0	107
In all...	967	416	16	1399

As it appears from the above table, I have divided the eye-colours mentioned above in three groups, viz:

1) **Blue** (i. e. simplex) comprehending such eyes as are indicated as pure blue, greyish blue, bluish grey and pure grey eyes.

2) **Brown** (i. e. duplex) comprehending such eyes as are indicated as brown, greyish brown, bluish brown, greyish brownish green, brownish green, greenish brown, yellowish brown and black.

3) **Greyish green** and **Bluish green** eyes, which I have kept in a group by itself, as it cannot be taken for certain whether they constantly belong to the simplex group or on the contrary

to the duplex one (the latter probably being most often the case). — The few families in which the parents were stated to have greyish green or bluish green eyes have not been taken into consideration.

Now we will examine whether the figures found may be said to enforce the theory hitherto in force. However, I must premise that the same importance cannot be attached to a material procured by means of questionnaires filled in by many different persons, as to such material as is procured by a single investigator by examination of the persons. Partly positive faults may slip in on account of some of the assistants not having made sufficient good investigations, so that faint brown pigment in an eye which is mainly blue has escaped their attention, partly there is another source of errors to which, however, that investigator is also exposed who makes his examinations in person, i. e. that in some cases it may be the question of illegitimate paternity. The greatest danger, however, is that the informant too much relies on his memory, even as regards long-dead persons. However, it cannot be doubted that the method notwithstanding is of value, when based on controlling enquiries. I hope this will appear from what follows.

Marriages: blue × blue. Pursuant to the theory no children with brown eyes could be born in such marriages. Apart from the 7 children of such marriages whose eyes are indicated as greyish green or bluish green and the positive value of which consequently is doubtful there remain 625 children with blue eyes and 12 with brown eyes. One might suppose from the exceedingly small percentage (less than 2 %) that it is a question of wrong statements. Having made a closer examination of the question I do not doubt, however, that the apparent deviations from the principal rule in certain cases are due to other conditions. As is well known, it is not unusual to meet persons whose right and left eye is differently coloured, or persons in whom the iris has a greater or smaller sector of another colour than that of the rest of the eye. As a person thus may have partly blue eyes and partly brown eyes, the possibility is not far that a person who genotypically has a dominant brown factor in certain cases may seem to have pure blue eyes, viz. in case the appearance of the pigment on account of a patological condition

in the eye is prevented — not locally at in the case mentioned above but completely, — so that the iris of both eyes is blue. It is beyond doubt that I have found such instances in my material. Thus I have two instances showing that where it was stated in the questionnaires that two parents with blue eyes had children with brown ones, it appeared on further enquiries that at the same time there was an inheritable disposition for abnormal vision, astigmatism, weak sight or the like. The cases were:

1) In the couple A. both parents had blue eyes and had 9 children, viz. 5 daughters and 3 sons with blue eyes and one son with brown eyes. On further enquiry as to the power of vision of the persons in question I received i. a. the following information: ". . . . The parents' eyes were even light blue with dull glass lustre. As was supposed both parents wore glasses: the mother's eyes were astigmatic and those of the father presbyope, and the son (with brown eyes — Ö. W.) at present seeks Dr. L. for glaucoma". I have not got any information as to the other sisters and brothers.

2) In the couple H. both had blue eyes: the husband had dark blué eyes, the wife light blue ones. They had 7 children of which 2 daughters and 2 sons with blue eyes, and one daughter and 2 sons with brown eyes. On further enquiry as to the power of vision of the persons in question, I got the following information of the husband, that he "had undergone an operation in Aalborg for glaucoma, that his vision was all right, but that he had to wear exceedingly strong glasses and . . . . of late years suffered very much from headache which, in the opinion of the physician, originated in the eyes". — 3 of the sons were stated to be myope, i. e. one with brown eyes and 2 with blue ones. One of the last mentioned, however, only wore glasses when reading.

The value of such instances of course cannot be estimated until it has been shown in how many cases no disease of the eyes is found in connection with the above mentioned deviations. Altogether I have 8 statements to the effect that parents with blue eyes have children with brown eyes, viz. in all 12 children with brown eyes. It does not interest us in this connection that originally I had a few other statements which on further investigation appeared to be owing to wrong observations.

In 2 cases out of 8 the addresses of the persons in question were unknown to me, thus a further enquiry could not be made. In one case the person concerned could not procure any information. In 2 cases out of the remaining 5 abnormities were found in the eye, and it was declared in 3 cases that no defects in the eye were found. One of these 3 cases is of special interest and well elucidated. It will be fully accounted for here.

In the couple H., a well-known Danish family,* both husband and wife had blue eyes. They had 12 children, viz. 7 daughters and 3 sons with blue eyes and 2 daughters with brown eyes. When I call these 2 daughters brown-eyed, it is because the iris in their eyes contained pigment of an obviously yellowish brown colour. I have personally examined one of these daughters and ascertained an ample quantity of brown pigment in both eyes. It is said of the other daughter: "It is beyond doubt that next to the pupil is found a narrow cohesive ring, the colour of which may be determined as yellowish brown. Besides there are further out on the iris, especially in the left eye, some greater or smaller yellowish brown spots or corns". Apart from these dots or corns her eyes are stated to be of a dark greyish green colour. In her marriage with a man with blue eyes she has 6 children, all with blue eyes, viz. one daughter and 5 sons. Thus we have an instance of a single generation having brown eyes without finding this quality in the preceeding or following generation nor being in connection with any abnormity as to the power of vision — just as if brown eyes could be recessive to blue eyes.

I am of opinion that the explanation must be sought in the fact that one of the parents in the marriage H. is genotypically brown-eyed, but at the same time has had a pigment-restrictive disposition, such disposition, however, not involving any abnormity in the power of vision. It will be easily understood that one of the daughters with brown eyes has children only with blue eyes, viz. 5 sons and one daughter, provided it is a question of a sex-linked inheritance, as will be shown later.

Captain M. Kofoed-Jensen has furnished me with an interesting instance of inheritance through 4 generations of one-sided defect of the eye partly in connection with pigment-restriction. One of Mr. K.-J.'s ancestors by the female line had one blue and one brown eye (the blue one with but little power

of vision). This woman's daughter had iron-grey eyes (one without power of vision). The power of vision of one of the latter's daughters who is said to have grey "cat's eyes" is also very small, whereas her brothers and sisters are highly myope. Finally in the fourth generation there is a woman (with iron-grey eyes) whose one eye is astigmatic, while her 3 brothers and sisters have normal power of vision.

Thus we may recapitulate that deviations from the rule that parents with blue eyes only have children with blue eyes have been shown in quite isolated cases. This is due to other pigment-restrictive dispositions and especially sometimes such as involve abnormities in the power of vision.

Marriages: blue×brown or vice-versa. How many children with brown and blue eyes respectively theoretically may be expected in such marriages are dependent on how many of the parents with brown eyes are homozygously brown-eyed and how many are heterozygously brown-eyed. This again is dependent on the proportional number of brown-eyed and blue-eyed persons in the population. As the number of persons with brown eyes is in absolute minority in Denmark — what is evident when comparing the number of children (in table 1) of parents with blue eyes (644) with the number of children of parents with brown eyes (107), only very few persons are homozygously brown-eyed, surely less than 3 % of the whole population. How this may easily be computed will be accounted for at the end of this chapter.

Most persons with brown eyes (more than 90 %) thus are heterozygously brown-eyed; consequently marriages between persons with blue and brown eyes respectively might be expected to give somewhat less than 50 % of children with blue eyes and somewhat more than 50 % of children with brown eyes. As shown in table 1, 317 children with blue eyes and 322 children with brown eyes are found in such marriages; thus on account hereof we cannot say that the combination blue×brown is not in conformity with what might be expected pursuant to the ruling theory.

Marriages: brown×brown. As more than 90 % of persons with brown eyes as mentioned above in this country are heter-

ozygously brown-eyed, marriages between persons with brown eyes in consequence hereof should usually give 3 children with brown eyes for each child with blue eyes. The few homozygous individuals will of course only have issue with brown eyes. Beforehand it might thus be expected that somewhat less than 25 % of the children would have blue eyes, somewhat more than 75 % brown eyes. This also is in full conformity with the figures virtually found, 25 children with blue eyes and 82 children with brown ones being born in such marriages, as shown in table 1.

Altogether it must be admitted that the conditions found according to this summary consideration is in no complete conformity with the ruling theory on the inheritance of the eye-colour in man as might be expected according to the size and probable value of the material. However, it seems to be beyond doubt that sometimes other pigment-restrictive factors may be found which manifest themselves as pathological conditions in the eye and which breaks the rule that parents with blue eyes always will have children with blue eyes.

Before proceeding to show that the theory nevertheless does not hold good, it might be of interest to state a small mathematical formula which I have laid down and which might often be useful when making such statistical investigations on inheritance.

A formula. When in a population one has to do with inheritable pecularities due to a single segregating essential difference where full dominance is found — such as is supposed to be the case as regards the eye-colour of man — one will, as is known, only find two types, in our instance the brown-eyed type and the blue-eyed one. From an inheritable point of view the individuals marked by the dominant qualities may be divided into two groups, the homozygotes and the heterozygotes who to all appearance cannot be separated. It is very often interesting to know how great a percentage may probably be ascribed to one type and how many to the other. Provided that no selection between the individuals takes place at crossing — viz. if in our case persons with brown eyes do not preferably marry persons with blue eyes e. t. c. — an easily applicable formula may

be laid down, which directly gives the probable percentage of the homozygously dominant type in the population.

Given is in the population:

a % of the recessive type, thus $100 \div a$ % of the dominant one.

100 + a ÷ 20 |/a thus will be the percentage of homozygously dominants in the population.

20 |/a ÷ 2 a will be the percentage of heterozygously dominants.

In table 1 we find 416 (= 30.1 %) persons with brown eyes and 967 (= 69.9 %) with blue eyes. **a** = 69.9 gives about 2.7 % homozygously brown-eyed individuals.

The following scale clearly shows how the percentage of the homozygously dominant type in a well mixed population is to the percentage of the recessive type and to the heterozygously dominant type.

In a population with

0 % recessives	are 100 % homozyg. dom.	0 % heterozyg. dom.
1	81	18
4	64	32
9	49	42
16	36	48
25	25	50
36	16	48
49	9	42
64	4	32
81	1	18
100	0	0

It appears from this scale — which by the bye is not anything new — that the heterozygously dominant type at most comprehends 50 % of the population and that the frequency per cent decreased whether the recessive type or the dominant one increases in number. It may be useless to state that the above calculation taken by way of example of the percentage of persons homozygously brown-eyed will be incorrect (i. e. still smaller) when a new factor for brown-eyedness — as shown in the following chapter — must be introduced.

III. On a sex-linked factor.

What made me make a closer analysis of my material was the fact that investigators as an absolute rule state that the number per cent of persons with brown eyes within each sex constantly is greater among women than among men.

On the basis of an investigation made by the Statistical Laboratory of the Copenhagen University in 1893, comprehending about 300,000 school-children, Sören Hansen (1909) states that there is a difference in the average degree of pigmentation in the eyes of boys and girls. After having shown that the degree of pigmentation calculated on a numerical scale increases with the age, he says (p. 304): "Provided this difference between the pigmentation of boys and girls may be considered as real — what is undoubtedly the case as far as the eyes are concerned — it is not far to couple it with the earlier development of girls. If this be the case, it may be said that the boys' hair and eyes on the average have the same degree of pigmentation as those of girls two years younger. To this must, however, be added that this difference cannot be looked upon as a secondary sexual character, as it has been proved by concurrent researches in other countries (Beddoe, Pfitzner) that the difference is considerably greater in adults than in children. In other words the deposit of pigment takes place to a much greater extent in women than in men and is continued far on in the years."

Sören Hansen finds that the relation is as follows as regards 1000 boys and girls between the ages of 6 and 14:

	Boys	Girls	In all
Blue eyes	628	621	1249
Brown eyes	372	379	751
	1000	1000	2000

The difference is not great but as the material in itself is enormous the difference, however, is past dispute. And moreover it is here a question of children in whom the difference is less distinctive. Besides it must be noted that the questionnaires sent out did not contain the indications: blue and brown eyes, but light, middle and dark eyes. Further guidance, however, was given the investigators (the teachers) in an accompanying explanation, from which it appeared that "middle" and

"dark" eyes should all contain brown pigment, whereas "light" eyes, if anything, meant blue eyes, light greenish eyes, however, being referred to this group.

The difference of the frequency per cent of brown eyes in men and women respectively will with greather explicitness be evident from the following statistics.

As regards Sweden Lundborg (1920) states as follows:

	Männer		Frauen	
	Hell %	Dunkel-meliert + Braun %	Hell %	Dunkel-meliert + Braun %
Schweden in Wärmland	79.0	21.0	71.4	28.6
Finnen in Norbotten	74.3	25.7	62.0	38.0
Lappen	26.6	73.4	20.3	79.7
Seminaristen	81.6	18.4	79.4	20.6
Tuberkulose-Patienten:				
15—24 Jahre	75.4	24.6	69.7	30.3
25—49 —	76.6	23.4	70.3	29.7
Landstreicher, Sträflinge und Verwahrloste, 15—24 Jahre	79.1	20.6	72.6	27.4

Lundborg further quotes the investigations made by others: E. g. he writes (p. 165): "Gelpe gibt für die Karlsruher Volkschulkinder 37 % dunkeläugige Knaben und 45 % dunkeläugige Mädchen an. Wiazemsky findet unter den Knaben und Jünglingen zwischen 10—18 Jahren in Bulgarien 62 %, unter den Mädchen im selben Alter 74 % braunäugige. Elkind gibt für polnische Juden (erwachsene Personen) 49.0 % (bei Männern), resp. 53.6 % (bei Frauen) an".

On p. 168 Lundborg writes: "Die Anzahl der Braunäugigen unter schwedischen Militärpflichtigen (44935 Soldaten) ist 4.5%". However, he does not include the "dunkelmelierten". P. 176: "Hätten wir Gelegenheit eine ebenso grosse Anzahl gleichalteriger Frauen zu untersuchen, würden wir bestimmt finden, dass wenigstens 6 % braune Augenfarbe haben. Analogieschlüsse zwingen uns zu einer solchen Annahme."

Halfdan Bryn (1902) examined 834 individuals in Selbu

and Tydalen in Norway. He divided his material into two parts according to the persons concerned being more or less than 16 years. He came to the following result:

	Men		Women	
Selbu:	"Simple"	"Double"	"Simple"	"Double"
Less than 16 years	63.9	36.1	56.9	43.1
More — —	63.0	37.0	49.3	50.7
Tydalen:				
Less than 16 years.....	53.6	46.4	51.8	48.2
More — —	49.4	50.6	36.5	63.5

As the author (p. 188) adds: "Concerning the eye-colour it is reasonably safe to assume that no mistakes are done in grouping the children in "simple" and "double" eyed when two or three years old", — a great part of the persons of less than 16 years examined by him must have been even less than 2 years, there being a discernible difference in the percentages between his "children" (less than 16 years) and "adults" (more than 16 years) — or a part of the children with blue eyes must have died, especially the girls with blue eyes, as a shifting towards blue-eyedness from one generation to the next in no way in these parts can be called forth by immigration.

As appears from the above the percentage of women with brown eyes is thus everywhere greater than that of men with brown eyes. But the question arises how this fact may be reconciled with the view that the qualities: brown-eyedness blue-eyedness segregate according to the simple Mendelian scheme. It is clear that at any rate these two things are not consistent and in case the question on the whole can be explained without taking into consideration the possibility of a sex-linked inheritance, there is a priori only one way out, viz. that the mortality among growing women with blue eyes is greater than among those with brown eyes, or that mortality among men with brown eyes is greater than among men with blue eyes.

As far as I know nothing, however, justifies the supposition that there is a difference in this respect in men and women, although statistics from time to time have been forthcoming which seem to show the difference in the death-rate of dark-haired and fair-haired persons respectively. Whether such statistics are correct, I do not know.

Under all circumstances one is necessarily led to suppose an heritably different vitality of individuals with brown and blue eyes respectively or the corresponding gametes. When considering my following statistics one must absolutely be compelled to take a more different view.

As mentioned above 648 persons are found in my material whose parents had different eye-colour, either the father having brown eyes and the mother blue ones or vice-versa. 317 of these persons had blue eyes, 322 brown eyes, while the eye-colour of 9 persons was difficult to determine. In case the material is divided into two groups according as the father or mother having brown eyes, one gets essentially different results, as shown in the following tables:

Table 2.

Mother blue × Father brown.

The eye-colour of the children	Sons	Daughters	In all
Blue	63	50	113
Brown	65	81	146
Greyish green-bluish green	4	2	6
	132	133	265

Table 3.

Mother brown × Father blue.

The eye-colour of the children	Sons	Daughters	In all
Blue	101	103	204
Brown	87	89	176
Greyish green-bluish green	0	3	3
	188	195	383

The tables speak for themselves but I want to fix the difference to mean that when the father has brown eyes the number of sons with blue and brown eyes respectively is almost alike, whereas there are many more daughters with brown eyes than with blue ones. If the mother has brown eyes, the number of both sons and daughters with blue eyes exceeds that of

children with brown eyes. The figures without doubt are so great that casualities cannot be the cause of the characteristic difference and so far the result cannot be wondered at, as the difference in the number of women and men with brown eyes must have a natural explanation.

The tables clearly show that marriages in which the husband has brown eyes are much less frequent than marriages in which the wife has brown eyes, 265 and 383 children respectively being born which corresponds to 65 and 91 marriages respectively.

In general one would gather from the tables that some of the 81 daughters with brown eyes in table 2 ought to have had blue eyes and perhaps they are only phenotypically brown-eyed. If this view was permissible, the result in table 3 would also be intelligible; for in case some of the women with brown eyes were genotypically blue-eyed, some of the marriages: mother brown × father blue must of course only give children with blue eyes; consequently such children would be in the majority, as is also the case.

Such an "explanation" was not, however, satisfactory, for the consequence hereof would be that the eye-colour was not in the true sense of the word genotypically determined and investigators of genetics have in view of other researches no reason to agree in such a supposition.

Thus we must look for a theory which is in conformity with the views taken by such investigators. That such a theory can be found will appear from what follows.

As is well-known sex-linked inheritance is found in man. The man appears to be heterozygous as regards the sex-linked inherited qualities — for which (e. g. reddish green allochromasia, hemophilia) it is the rule that generally only men show this quality (the disease), whereas women transmit it without showing the symptoms themselves. In conformity herewith Winiwarter is of opinion that he has found 47 chromosomes in men, wherefrom the conclusion may be drawn that women must have 48. Thus men have one sex-chromosome (XO), women two (XX). Consequently men form 2 kinds of genital cells, with 23 chromosomes and 23 + 1 chromosome respectively while women only form one kind of eggs, i. e. such eggs as have 23 + 1 chromosomes.

The theory by which I shall try to explain the conditions of inheritance of the eye-colour, is that we have to do with 2

factors, of which one — in conformity with the assumption of previous investigators — has its seat in a pair of autochromosomes, thus involving a general Mendelian segregation, whereas the other new factor has its seat in the heterochromosomes, thus entailing sex-linked inheritance.

One factor which I call B (allelomorf: b) is the dominant brown factor in the pair of autochromosomes; the other which I call W (allelomorf: w) is the dominant brown-factor found in the pair of sex-chromosomes. Both factors severally or between them give pigmented eyes (duplex) in both men and women. There is nothing particular in this, but what I am obliged further to surmise is, that bW eggs are unable to exist, that bW gametes are exterminated in women.

These suppositions in view the question may be explained — or rather the latter supposition in view, as it is no free supposition that sex-linked inheritance takes place, but it is an indisputable result of the investigations of the reciprocal connections.

Let us consider all possible genotypical constellations according to the above theory:

Men		Women	
BBWO, BbWO	with brown eyes	BBWW, BBWw	with brown eyes
BBwO, BbwO	with brown eyes	BBww, BbWW, BbWw	with brown eyes
bbwO	with blue eyes	Bbww, bbWw	with brown eyes
		bbww	with blue eyes

Thus there are 5 formulae for men, of which 4 brown-eyed, and 8 formulae for women, of which 7 brown-eyed. That the formula bbWO cannot exist in men is a consequence of my above supposition that bW eggs are not capable of living, as the formation of the zygote bbWO beside a male gamete bO also requires a female bW. From the same reason the formula bbWW cannot either exist in women.

In order to control whether the theory is in conformity with the actual conditions, we must remember — what has been shown above — that persons with brown eyes in Denmark almost always are heterozygously brown-eyed. In order not to

make the calculations too great, we may without running the risk to dislocate the figures essentially only consider the results of crossing between the heterozygous types and not take into consideration such types in which one or both factors are found double. The miscalculation will give us a somewhat too small number of persons with brown eyes in the issue, but it is only a question of a few per cent.

Thus we only consider the following types:

Men		Women	
BbWO, BbwO	with brown eyes	BbWw, Bbww, bbWw	with brown eyes
bbwO	with blue eyes	bbww	with blue eyes

First we try to calculate the appearance of the offspring by the combination: men with brown eyes and women with blue eyes. The men with brown eyes belong to 2 types, BbWO and BbwO, i. e. partly that with the W-factor, partly that without such factor. The calculations made by me show, that the W-factor must be supposed to be found only in very few men with brown eyes, viz. in about 20 % of men having the formula BbWO; the rest, about 80 %, has the formula BbwO.

In the following table (no. 4) will be seen the formula for and the number of the types of gametes active at this crossing as also the formulae of the offspring.

Table 4.

Brown ♂ { BbWO — 20 %, BbwO — 80 % } × Blue ♀ bbww — 100 %

20 BbWO	80 BbwO	♂ gametes	♀ — gametes 100 % bw	
5		BW	5 BbWw	= 5 brown ♀
5	20	BO	25 BbwO	= 25 brown ♂
5		bW	5 bbWw	= 5 brown ♀
5	20	bO	25 bbwO	= 25 blue ♂
	20	Bw	20 Bbww	= 20 brown ♀
	20	bw	20 bbww	= 20 blue ♀

This crossing should thus give the following result:

	♂	♀
Blue	25	20
Brown	25	30

The confirmity with what is actually found will be seen from the following table

	♂		♀	
	Blue	Brown	Blue	Brown
Actually found	63	65	50	81
Theoretically expected	64	64	52.4	78.6

As will be seen the percentage theoretically expected are in full confirmity with the number actually found.

We shall then (vide table 5) try to make a similar calculation of the reciprocal crossing, as — through the above table 4 — we have obtained information of the relative frequency of the different formulae of women with brown eyes.

Table 5.

Brown ♀ { BbWw (5 =) 16.67 %
bbWw (5 =) 16.67 % × Blue ♂ bbwO — 100 %
Bbww (20 =) 66.67 % }

16.67 BbWw	16.67 bbWw	66.67 Bbww	♀-gametes	♂-gametes 50 % bw	50 % bO	
5.56			BW	2.78 BbWw	2.78 BbWO	= 5.56 brown
5.56		33.33	Bw	19.45 Bbww	19.45 BbwO	= 38.89 brown
5.56	16.67	33.33	bw	27.78 bbww	27.78 bbwO	= 55.56 blue

In table 5 consideration has been taken to the fact that the bW eggs cannot exist; thus only 3 kinds of eggs are formed,

BW, Bw and bw with the frequency per cent as shown in table 5. It will appear from the table that 5.56 i. e. 38.89 % of persons with brown eyes will come forth (as many ♂ as ♀) and 55.56 % with blue eyes (as many ♂ as ♀). Or in other words:

	♂	♀
Blue.....	27.78	27.78
Brown	22.22	22.22

The conformity with what is actually found will be seen from the following table:

	♂		♀	
	Blue	Brown	Blue	Brown
Actually found	101	87	103	89
Theoretically expected	104.5	83.5	106.7	85.3

Also in this case the number actually found is in conformity with the number expected pursuant to my theory. Both here and in the reciprocal crossing a few more persons with brown eyes are actually found than calculated.

As, however, we have excluded the homozygously brown-eyed persons — the number of such persons being very small — it is only natural that the deviation is in this direction.

Finally, if we want to control what the crossing: brown × brown according to the theory will give, we will find this by calculating the following combinations:

Table 6.

Brown ♂ { BbWO — 20 % / BbwO — 80 % } × Brown ♀ { BbWw — 16.67 % / bbWw — 16.67 % / Bbww — 66.67 % }

♂-Gametes	♀-Gametes (per cent)		
	5.56 BW	38.89 Bw	55.56 bw
5 BW	0.28 BBWW	1.94 BBWw	2.78 BbWw
25 BO	1.39 BBWO	9.72 BBwO	13.89 BbwO
5 bW	0.28 BbWW	1.94 BbWw	2.78 bbWw
25 bO	1.39 BbWO	9.72 BbwO	13.89 bbwO
20 Bw	1.11 BBWw	7.78 BBww	11.11 Bbww
20 bw	1.11 BbWw	7.78 Bbww	11.11 bbww

By summing up the number of persons with brown and blue eyes respectively we will find:

	♂	♀	
Blue.....	13.89	11.11	25
Brown	36.11	38.89	75
	50	50	100

Thus:

	♂		♀	
	Blue	Brown	Blue	Brown
Actually found.....	15	42	10	40
Theoretically expected (among 57 ♂♂ and 50 ♀♀)	15.8	41.2	11.1	38.9

Also in this case the conformity is almost peculiarly good: thus we may say that this theory in case it is acceptable in other respects is very probable also, on account of this good conformity.

However, I must state that I do not consider the value of the theory as finally proved as regard the details.

The theory is somewhat "artificially" adapted to the material before me, but it is a matter of course that it cannot be otherwise. However, it would not be difficult to control the value of my results by undertaking a similar examination as that made by me, especially as regard offspring of parents with different eye-colour. Ophtalmologists e. g. would easily be able to gather a valuable material for this purpose.

As to the instance mentioned on p. 5—6 in which out of 12 children of parents with blue eyes 2 daughters had brown pigment in the eyes, it was probably the sex-linked W-factor which brought about the eye-pigment, as one of these daughters, probably of the formula bb Ww, in her marriage with a man with blue eyes has 6 children all with blue eyes. A woman of the said genotypical constitution will produce only bw-eggs, as the bW-eggs, as we know, are not capable of living.

As will be understood my theory does not essentially alter what was originally stated by Hurst and Davenport, viz. that blue-eydness (simplex) is recessive towards brown-eyedness (duplex). I am only obliged furthermore to reckon on a sex-linked brown factor as an explanation of the frequency of brown-eyedness among men and women and as an explanation of the reciprocal combinations of parents with different eye-colour giving different results.

According to this theory two blue-eyed parents will not have children with brown-eyes, and when in my material I have been able to show that the deviation among the few certain cases where the rule does not keep sometimes at any rate must be due to abnormities in the building of the eye, and that it was only possible in two families to show with certainty that no eye-disease might have caused the deviation, I do not doubt that we might say that two parents with blue eyes will only have blue-eyed children, unless one or both of the parents are pathological, which generally shows itself in abnormal vision.

Left are now Bryn's 4 instances which have been previously mentioned, that blue-eyed parents have children with brown eyes. In these cases nothing was stated as to the power of vision. It is contrary to the results of other investigators that Bryn finds that of 4 out of 30 marriages between persons with blue eyes, children with brown eyes are born; it would be worth while to follow the matter in order to ascertain whether in the Norwegian towns, where Bryn has undertaken his investigations, more general inheritable dispositions are found, which effect the eye-colour — or whether it is a question of rare phenomena which must range under the pathology.

I must, however, observe that I fully realize that the indication "pathological" for such rare persons does not give any satisfactory explanation of the nature of these exceptions. Partly the possibility exists that a purely physical influence during the development of the individual may prevent the formation of pigment in a genotypically brown eye, and partly the possibility exists that something inheritable may form the basis of the deviation. Both possibilities are perhaps found in Nature, and it especially appears from my cases that certain inheritable abnormities in the power of vision may prevent the appearance of the pigment. This further complicates the matter as it shows that besides the two pair of factors already taken into consideration — of which one has its seat in the autochromosome pair, the other in the sex-chromosomes — we have also to reckon with other, though much rarer factors, especially such as influence the power of vision.

As is well-known, the question of the mode of inheritance of the eye-colour has a certain practical importance, viz. where it is a question of determining paternity. Although according to the investigations made I do not consider it permissible quite to deny that parents with blue eyes may have a child with brown eyes, it appears on the other hand that when both parents have a normal power of vision, it is so exceedingly rare that it may be said to be of considerably practical importance in cases of paternity to have knowledge of the eye-colour.

Résumé.

About 1400 children and their parents form the basis of this investigation.

While it is the main rule that blue eye-colour (simplex) is recessive towards brown (duplex), exceptions are now and then found from the rule that blue-eyed parents always will have blue-eyed issue. These exceptions originate in the presence of pigment-restrictive dispositions in one of the blue-eyed parents, sometimes in the presence of factors which besides preventing the formation of pigment show themselves in the abnormal power of vision of the person in question.

The fact that brown-eyedness among women everywhere is more common than among men is due to the fact that besides the Mendelian factor which has long ago been recognized, another dominant brown factor is found (W) which has sex-linked inheritance. In conformity with this supposition marriages between a woman with blue eyes and a man with brown eyes will give issue of quite another combination than the reciprocal connection. In the first case an equal number of sons with blue and brown eyes respectively are found in my material but more than one and a half time as many daughters with brown eyes as daughters with blue ones. In the latter case there is a surplus of blue-eyed offspring both among sons and daughters.

The statistics resulting from my investigation will be understood from the supposition of the above two factors, viz. one in the pair of autochromosomes (B) and one in the sex-chromosomes (W) as also the supposition of the elimination of all bW-eggs.

I express my thanks to the Trustees of the Carlsberg Fund who has enabled me to carry out these investigations as also to the many persons who have taken the trouble to fill in the questionnaires which form the basis of my work and to answer further inquiries directed to them. Finally I thank stud. mag. Bengt E. Dahl who has assisted me with the statistical treatment of the questionnaires.

Carlsberg Laboratory, Copenhagen, March 8, 1921.

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COLORIMETRIC DETERMINATION OF THE CONCENTRATION OF HYDROGEN IONS IN VERY SMALL QUANTITIES OF BLOOD BY DIALYSIS.

BY

J. LINDHARD.

Dale & Evans[1]) have lately published a method, by means of which it is possible to determine the reaction of blood by dialysis. In their experiments the two authors make use of about 1 cc. of blood, which accordingly must be obtained by puncture of an artery or a vein. Such samples of blood can of course be taken both once or twice, also from human beings, without any ill effects; but if it is desired to make a series of such determinations within a short time, e. g. before and after muscular exercise, the method will doubtless be found to entail considerable trouble. It will technically be very difficult to puncture a vein not to speak of an artery, when the subject cannot keep quiet; and one dare not let a cannula stay in the larger blood-vessels during the exercise on account of the danger of abrasion to the walls of the said vessel caused by the frequent twitching of muscles always accompanying heavy bodily work. It will therefore be of no small interest to find a modified form of this method making it possible to perform a determination of the reaction after the principle of Dale & Evans with such small quantities of blood that the sample may be taken from the capillaries in the usual way by means of puncture in a finger or in the ear.

The experiments of which further particulars are given below have shown this to be possible.

The apparatus consists of a small collodion-bag or thimble (a), mounted on a short piece of slightly conical glass tubing (b)

[1]) The Journal of Physiology 1920, **54**, p. 167.

to which it is fastened by means of a thin walled piece of rubber tubing (c). The glass tubing is closed at the upper end by means of a small cork (d). The mounted bag is put into a dialysing vessel (small test tube) (e) of a size corresponding to the dimensions of the bag so that the rubber tubing serves as a stopper (Fig. 1). For the colorimetric comparison test tubes of the same diameter are utilized. The dimensions employed were as follows: The bags were formed over a glass-rod 4 mm. thick; bag and tubing together up to the stopper would contain about 0,25 cc. The exterior diameter of the dialysing vessels was about 7 mm. and their length 25 mm.

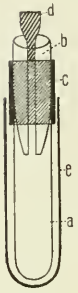

Fig. 1.

The collodion bags. The permeability of the bags depends on their thickness and the time of drying, but mostly on the collodion used. It is difficult to fasten very thick bags on the conical glass tube, and when mounted they are not easy to arrange in the dialysing vessel; moreover they seem to possess less permeability than the thinner bags which have been dried the same length of time. Very thin bags are more easily placed but are very difficult to change; they are easily spoilt in cleaning, especially if the blood is coagulated. As, however, even the best bags only can be used twice or thrice for blood, and as the bags are easily made, when one has suitable material to hand and is accustomed to the technique, it will probably be the best plan only to use the same bag once. As for the time of drying the permeability is largest with the shortest time of drying, and the difference shows the more clearly the thicker the bags.

The consistency of the collodion used may be varied by diluting it with alcohol and ether in different proportions. The suitable dilution just as the thickness of the bags and their time of drying must be found by direct experiment. The collodion used for these experiments has been made in the laboratory (the ordinary collodion of the trade is generally not suitable) of 14 gr of collodion cotton (also made in the laboratory) dissolved in 400 cc. of absolute alcohol and anhydrous ether in the proportion of 3 to 1. This solution is again diluted by an equal volume of alcohol and ether (3 : 1). Then the bag is made in the following way: You dip a glass rod of 4 mm thickness and with a rounded end into the collodion. It is then slowly raised so that

most of the collodion drains off. The dipping is repeated several times (e. g. 5) in the same manner, according to the thickness the bag is intended to have; and the layer of collodion is then allowed to dry in the air for 3 to 5 minutes, whereupon the whole is placed in water for a few minutes before the bag is loosened. After being loosened the bag is immediately put in water.

The bags can absorb Hæmoglobin which appears as yellowish spots and stripes, without, however, apparently influencing the permeability. The bags will also absorb the indicator used, which accordingly must not be added to the outer fluid till the bag has been removed.

The permeability of the bags is tested with S. P. L. Sorensen's Phosphate Mixtures, which for this purpose must be taken from the same mixture with which the corresponding test tube in the colour scale is filled, and an absolute colour match must be insisted on between the colour of the dialysate and that of the phosphate mixture from the scale. If this is not attained — even after repeated experiments — the bag must be discarded. In these experiments I have fixed the time of dialysis at 30 minutes. Dale & Evans have found that the dialysis could be finished in a shorter time. I have, however, not particularly tried to determine the shortest time possible, but have examined the bags I found suitable, with a view to determine how long a time it really took to get a complete dialysis so that the colour of the dialysate exactly matched that of the phosphate mixture used, with direct addition of the indicator. The following experiments may serve as examples:

Phosphate Mixture $P_H = 7.35$:	I	II	III	IV
Dialysis in Minutes[1] ..	30	25	19	15
P_H	7.35	7.30	7.35	7.32
Dialysis in Minutes	16	20	25	30
P_H	7.35	7.32	7.35	7.35
Dialysis in Minutes[2] ..	30	25	20	16
P_H	7.35	7.35[3]	7.33	7.30

[1]) Bag and tube quite full of Phosphate Mixture.

[2]) Bag and tube filled with about 4 drops of Phosphate Mixture and then filled up with $^n/_{10}$ Na Cl.

[3]) New bag.

Defibrinated Blood:				
Dialysis in Minutes	30	37	40	50
P_H	7.25	7.25	7.25	7.25
Phosphate Mixture $P_H = 7.25$:				
Dialysis in Minutes	23	24	25	27
P_H	7.25	7.25	7.25	7.25
Defibrinated Blood:				
Dialysis in Minutes	50	78	40	30
P_H	7.22	7.25	7.25	7.25

With regard to bag I 16 minutes is apparently sufficient; bag IV is, however, not trustworthy if the dialysis lasts less than 20 minutes. The reason for this may be that bag I was the largest and IV by far the smallest of all the bags used. To be on the safe side I therefore decided on a time of dialysis of 30 minutes in all cases. According to the results of the last experiments mentioned I think that it would be to no purpose to use a longer time.

As Colour Indicator I have used a 0.01 p. c. solution of Phenolsulfonephthalein, the changing range of which is just within the P_H values one can expect to meet with when working with blood, and which gives much greater colour changes by small differences in the concentration of hydrogen ions than for instance neutral red. As titration with the indicator seems to me very inexpedient, and, when we have to do with such small quantities of fluid as here, rather impossible to perform, I have by means of S. P. L. Sørensen's Phosphate Mixtures made up a colour scale, the intervals of which correspond to a difference of 0.05 in P_H. It is then easy to see if a certain test is within one or the other interval, and after some training one can, not only judge which of the colours of the scale is nearest to the test, but also how near, so that the reading may in the best case be performed with an accuracy of 0.01, i. e. by a trained investigator, in a good light, when the colours are seen against a white background. The reading is most difficult, when the colour of the test just corresponds to the colour of one of the mixtures in the scale, as then the colour of the test on account of the contrast will seem too yellow when placed on the red side of the test tube from the scale, and too red if placed on the other. In this case one must compare the two samples alone quite apart

from the scale. The colour scale must be renewed daily. It does not change perceptibly in a few hours; but in a couple of days it may change rather much, so that the error can reach 0.05 to 0.1 in P_H, and the direction of the change is towards the acid side. The bleaching of the colour scale may be due partly to an absorbtion of carbon dioxide from the air, partly to a change of the colloidally dissolved colouring matter. When the fluid is covered with a thin layer of oil of paraffin, the bleaching seems to become less, though not always.

When working with dialysates of blood one must take care that the dialysate does not give off any carbon dioxide to the air, till the colour comparison has taken place. I tried to obtain this by covering the dialysate with toluol; by putting a little toluol in the bag and in the capillary pipette with which the blood was absorbed, I hoped also to be able to prevent coagulation. The toluol did not give trouble in the dialysing process, but it was proved that a dialysate covered with toluol changed its colour in the alkaline direction more quickly than when it could freely give off carbon dioxide to the air. As the colouring matter cannot be dissolved in toluol or affected by this substance one must suppose that there were some substances in the dialysate which could be dissolved in toluol, and the removal of which made the dialysate more alkaline, e. g. fatty acids. It was proved by experiment that a solution of soap added to the indicator very soon became red when covered with toluol. Benzol behaved in about the same way. But by covering the dialysate with oil of paraffin it is at any rate possible to retard the giving off of carbon dioxide to the air very much.

As Outer Fluid I used a 0.1 n solution of chloride of sodium. The quantity of the outer fluid was generally 0.15 to 0.20 cc. The whole volume of bag and tube together till the stopper was as before mentioned 0.25 cc. It was proved to be of no consequence whether the inner fluid was pure phosphate mixture, or consisted of 3 or 4 drops of this mixture diluted with the solution of chloride of sodium, as this dilution is equivalent to an increased quantity of the outer fluid, which is of no consequence with the quantities of fluid and within the range of reaction met with. If in 5 test tubes one puts 1 cc. of 0.1 n solution of chloride of sodium, and then adds respectively 1, 2, 3, 4, 5 drops of the phosphate mixture, one cannot, after adding the same

quantity of the indicator to all the samples, observe any difference in colour. If a solution of bicarbonate of soda is substituted for the phosphate mixture a corresponding result will be obtained, in conformity with some earlier experiments (Jessen-Hansen) not yet published showing that dilution did not influence the result when the reaction was near the neutral point.

The determination of the reaction of blood takes place in the following way: The bag is emptied of the saline solution in which it has been kept, by means of a capillary pipette, and a small quantity of hirudin is put in to the bottom of the bag. A large drop of blood is quickly pressed out from a prick in a finger and then absorbed by the capillary pipette, containing a trace of hirudin solution. The blood is sucked in quickly from the interior of the drop, and a little blood is left so that the next drop is pressed out under it. Three large drops are enough to fill the bag and sometimes also part of the glass tube. The blood is added from the bottom of the bag in order to avoid air bubbles. As soon as this is done, the bag and glass tube is quickly filled up with a 0.1 n solution of chloride of sodium from another capillary pipette, which must be ready filled beforehand, and the stopper is put in place. After having wiped the bag and tube so that no blood remains on them — as some of the fluid may escape, especially when placing the stopper — the whole is placed in the dialysing vessel, which already is filled to the brim with a 0.1 n solution of chloride of sodium, in order to avoid the formation of air bubbles, and the whole is put aside for dialysis.

The addition of hirudin does not alter the concentration of hydrogen ions (experiments with phosphate mixtures and defibrinated blood from horses); but even the coagulation of the blood in the bags does not seem to alter the concentration appreciably. The space between the bag and the conical part of the glass tube reaching down into the former, will always be filled with the fluid (a 0.1 n solution of chloride of sodium or distilled water) in which the mounted bag has been kept; it is, however, very difficult in filling the bag to avoid leaving a small air bubble at the mouth of the glass tube, but this is of no consequence with regard to the determination. The bag must fit tightly on to the glass tube before the putting on of the rubber tube: if that is not so, some blood may, when this is

pressed down tight into the dialysing vessel, escape into the outer fluid between the bag and the tube, which naturally makes colorimetry impossible. The rubber tube must close the dialysing vessel tightly, if it is put in loosely or is not quite sound, the dialysate will give off carbon dioxide to the air, and the reaction will accordingly become too alkaline. A trustworthy result can only be obtained when the processes described are done quickly and without hesitancy.

If one is working with defibrinated blood the procedure is the same as mentioned, with the exception that all the blood with which the bag is filled is taken up into the capillary pipette at the same time. Phosphate mixtures not giving off carbon dioxide do not therefore require so much care; but in order to obtain the necessary quickness and precision it is recommended to use the same method when working with these as when making experiments with blood. Solutions of bicarbonate with addition of carbon dioxide are, however, very difficult to manage, and they are therefore very suitable to work with in order to obtain the necessary manual proficiency.

The colour scale may be made during the time the dialysis is in progress. The test tubes are filled with phosphate mixtures to the height of the dialysing vessels, and a suitable amount of indicator is added so that the colour differences appear as clearly as possible, and so that the concentration is as nearly as possible the same in all the vessels, which is not difficult after some training. The test tubes of the scale are placed on a stand so that they may easily and quickly be changed and that the colour is seen against a white background.

When the dialysis is finished the bag is removed, and the dialysing vessel is then quickly filled with a 0.1 n solution of chloride of sodium (which must be lying ready in a suitable pipette) so that there is just left room enough for the necessary quantity of the indicator. When this has been added and the vessel is closed with a (clean) finger, a tiny air bubble shall be found above the fluid by means of which the indicator and the dialysate are mixed by turning the vessel a few times. As soon as the mixing has taken place the fluid is covered with a drop of paraffin oil, and then the comparison with the colour scale is made.

The addition of chloride of sodium is naturally superfluous

when experimenting with phosphate mixtures, but even in this case it has the advantage that it is easier to add just the correct quantity of the indicator to the filled vessel than to a varying quantity of the dialysate in the bottom of it. In working with fluids containing carbon dioxide the air filled space above the dialysate will cause a smaller or larger systematic error in the result, which is shifted in the alkaline direction when the carbon dioxide is given off to the air. With regard to blood, the error seems to become rather constant and thus of less consequence. The experiments mentioned below with defibrinated horseblood gave constant results, well in conformity with the electrometric determinations, when the indicator was added to the dialysate and the vessel then turned round twice to ensure a thorough mixing. If the vessel was then closed again and turned round a few times, the colour became altered in the alkaline direction. In experiments with solutions of bicarbonate with carbon dioxide added, even one turning of the vessel shows an appreciable colour change, and the error is not only large but also varying much, as small variations from the accustomed technique show themselves clearly. The following experiment gives an idea of how large the error in question may be and also proves the value of the filling up of the vessel with the solution of chloride of sodium: One of the test tubes of the scale is filled with a bicarbonate solution, the indicator is added and distributed by means of a tiny air bubble; found $P_H = 7.28$. Another tube is filled to about one third, the indicator is added and the tube turned a few times; found $P_H = 7.41$. Then a test tube is filled with a bicarbonate solution as before; another is filled to about one third with the same solution, but then it is quickly filled up with a 0.1 n solution of chloride of sodium: the indicator is added and distributed by means of a tiny air bubble: both vessels give now $P_H = 7.28$.

In a few cases it has happened that the colour of the dialysate in a short time (10 to 15 minutes) changed appreciably in the acid direction; and so far it has not been possible to find a satisfactory explanation of the phenomenon. On one occasion the change mentioned took place with a phosphate mixture and it could be seen that the dialysate at first had the correct colour, thus proving that the unknown cause was not at work during the dialysis. Even if the error mentioned is only found except-

ionally one ought always to make sure against any mistakes by immediately performing the colour comparison.

As to the accuracy to be expected by the method I shall mention the following facts. The P_H values given below were found by colorimetric determination of a series of phosphate mixtures made up by another worker from the standard solution of primary and secondary phosphates according to the S. P. L. Sørensen Graph.

From Graph.	Colorimetry.
7.23	7.23
7.29	7.32
7.17	7.19
7.25	7.25
7.20	7.18

If, however, the dialysis is made with the same phosphate mixtures as have been used for the making of the colour scale, the correspondence becomes almost absolute as shown by the following examples. The tests have not only been compared with the colour scale but also with one another.

29/12 . . .	**7.25**	7.25	7.25	7.25	7.25	
26/1	**7.30**	7.30	7.30	7.30	7.30	
9/2	**7.30**	7.29	7.30	7.30	7.30	7.30
22/2	**7.30**	7.29	7.30	7.29	7.30	

In a series of experiments with defibrinated horseblood the colorimetric measuring was controlled by electrometry. The reaction of blood was varied by the addition of carbon dioxide. The examples given first show the mutual correspondence between the colorimetric tests, the table following gives the results of the comparative measurements. With regard to the colorimetric determinations the figures are either single determinations or averages of up to four experiments; among the electrometrical determinations there are two double determinations, the remainder are single determinations.

	Colorimetry.				Electrometry.	
26/11 . . .	7.37	7.38	7.34		7.37	7.41
29/11 . . .	7.34	7.32	7.33	7.33	7.38	
3/12 . . .	7.43	7.45			7.52	7.52
6/1 . . .	7.58	7.58	7.58		7.59	

	Colorimetry.	Electrometry.	Difference.
	7.35	7.32	+ 0.03
	7.23	7.15	+ 0.08
	7.10	7.11	— 0.01
	7.05	7.08	— 0.03
	7.15	7.19	— 0.04
	7.36	7.39	— 0.03
	7.33	7.38	— 0.05
	7.44	7.52	— 0.08
	7.58	7.59	— 0.01
Averages ...	**7.29**	**7.30**	— **0.0155**

The averages show a very good agreement between the two methods, but a glance at the differences proves, that rather large differences are occasionally met with. This uncertainty must be considered as due to the fact that none of the two methods could prevent the giving off of carbon dioxide from the blood during the measurements. The dialysing vessels were at that time not filled up with sodium chloride and loss of CO_2 must therefore take place when the vessels were turned to mix the dialysate and the indicator. An air space varying in volume was also found in the electrode vessels used, and would no doubt take up carbon dioxide from the blood during the experiment. When the results, nevertheless, showed such good conformity as they did, the reason must be that blood gives off carbon dioxide at a slow rate only.

To test this point further a comparison was made between the colorimetric and the electrometric determination of a solution of bicarbonate with added CO_2. The colorimetric determination took place in two ways, partly by dialysis with the usual technique, partly direct, when a test tube from the scale was quickly filled with the bicarbonate solution, after which the indicator was added and the fluid covered with a drop of paraffin oil. The results were:

	Colorimetry							
	direct		after dialysis			Electrometry		
$^7/_1$...	7.36—7.48	7.42	7.45	7.55	7.50	7.70	7.75	7.87

The sample for the first direct testing was taken before, that for the second after the samples for dialysis. The samples for electrometry were taken together with these; care was taken not to have the bottle with the bicarbonate solution open longer than

necessary. The great difference between the two direct tests is probably due to the fact that the second test tube was not sufficiently filled.

The result of the experiment is, that the manipulations, needed for dialysis cause a considerable loss of carbon dioxide, further that the electrometry was quite useless as control, the air spaces in the electrode vessels taking up large quantities of carbon dioxide. The air spaces in the electrode vessels were in the three tests 5.0—5.85—7.0 cc. respectively. When determining the P_H value for the air space 0 by extrapolation one gets $P_H = 7.32$: a value not very different from that of the first direct colorimetric test. As this direct test can be made practically speaking without any loss of carbon dioxide, when performed sufficiently quickly, and the vessel is quite filled, one may dispense with the electrometric control, because a comparison between the direct tests and the dialysates will supply the information wanted about the magnitude of the error caused by the loss of carbon dioxide.

	Dialysis				Direct tests	
$^{7}/_{1}$	7.42	7.45	7.55	7.50	7.36	7.48
$^{8}/_{1}$	7.40	7.38	7.38		7.31	7.32
—	7.40	7.38	7.35	7.37	7.28	7.27
$^{10}/_{1}$	7.28	7.31	7.28	7.32	7.18	7.19

	Dialysis	Direct tests	Differences
	7.48	7.42	+ 0.06
	7.39	7.32	+ 0.07
	7.375	7.275	+ 0.10
	7.30	7.19	+ 0.11
Averages ...	**7.385**	**7.30**	+ **0.085**

The dialysing experiments show that after some training, the error becomes quite systematic and increases with increasing carbon dioxide pressure. The error may, however, be diminished very much, if the air space is eliminated, as mentioned above, by filling the dialysing vessels completely with 0.1 n sodium chloride.

	Dialysis				Direct tests	
$^{10}/_{1}$	7.30	7.31	7.29	7.28	7.28	7.27
$^{11}/_{1}$	7.15	7.18	7.19		7.12	7.14
$^{13}/_{1}$	7.27	7.28	7.33		7.24	7.24
$^{15}/_{1}$	7.31	7.30	7.33	7.32	7.27	7.28

	Dialysis	Direct Tests	Differences
	7.295	7.275	+ 0.02
	7.17	7.13	+ 0.04
	7.29	7.24	+ 0.05
	7.315	7.275	+ 0.04
Averages ...	**7.27**	**7.23**	+ **0.04**

The variation within a single series of experiments is apparently of the same magnitude as in the previous experiments, but the systematic error is only half as large. When we remember that carbon dioxide is given off considerably more slowly from blood than from the bicarbonate solution, one may, I think, conclude from these experiments that the error in P_H due to the giving off of carbon dioxide to the air becomes considerably less than 0.04 when working on blood with the same technique. But the fact that the blood when taken from a puncture in a finger or an ear, must be sucked up drop by drop into the capillary pipette introduces another source of error, which cannot be directly controlled; when, however, the blood is sucked up in the way described above, viz.: that not all the blood pressed out is taken, but the outer layer is left, one may doubtless be sure that the loss taking place from the drop will at any rate be very small.

The 5 series of experiments mentioned below show the magnitude of the accidental error in experiments to determine the reaction of blood in the manner described above.

Blood from a finger:

$^{11}/_{2}$ ...	7.33	7.35	7.31	7.35	7.35
$^{16}/_{2}$ ...	7.30	7.37	7.34	7.32	7.34
$^{18}/_{2}$ ...	—	7.30	7.32	7.32	7.31
$^{21}/_{2}$ ...	7.34	7.35	7.34	7.35	7.31
$^{22}/_{2}$ ...	7.36	7.37	7.36	7.34	7.33

After calculation of the averages and dispersion for each series, we get:

n	A	μ
5	7.34	0.017
5	7.33	0.026
4	7.31	0.010
5	7.34	0.017
5	7.35	0.017,

which gives us $\mu = 0.021$ as the dispersion for the whole material. If all the experiments are considered as repetitions, which probably is not permissible, as the reaction of blood certainly is not absolutely constant from day to day, one finds the same dispersion. As the average of all the single determinations we find $P_H = 7.336$. As the average for P_H with a carbon dioxide pressure of 40 mm. in human blood generally given is 7.33 the systematic error in the determinations must also have been reduced to a minimum. The person experimented on has, however, an abnormally low alveolar carbon dioxide pressure, and it is therefore possible that a small systematic error in the alkaline direction may be present in the results. But this cannot of course invalidate the method to any appreciable extent.

I beg to tender my cordial thanks to Professor Dr. S. P. L. Sörensen, who has helped me in working out the method, for the interest he constantly has shown in my work. I also beg to thank Mrs. S. P. L. Sörensen who has helped me with the making of the collodion bags, and the assistant Mr. Linderström-Lang, graduate of the Royal Engineering college, who has made the electrometric determination, and also in different ways helped me during my work at the laboratory.

The Carlsberg Laboratory, March, 18 1921.

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SUR L'«ERREUR DE SEL» INHÉRENTE À L'ÉLECTRODE DE QUINHYDRONE

PAR

S. P. L. SØRENSEN, MARGRETHE SØRENSEN ET K. LINDERSTRØM-LANG.

A l'occasion de la réunion tenue l'été dernier pour célébrer le centenaire de H.-C. Oersted, M. le Dr E. Biilmann, Professeur à l'Université de Copenhague, a fait une conférence dans laquelle il faisait connaître un procédé nouveau pour la mesure des concentrations en ions hydrogène. M. Biilmann a plus tard complété cette communication par quelques remarques faites dans la Société Chimique de Copenhague, et enfin il a publié un exposé complet de sa méthode dans l'écrit de fête (Universitetets Festskrift) que l'Université a fait paraître en novembre dernier.

Cette méthode nouvelle se base sur un fait signalé par son auteur, à savoir qu'une solution aqueuse acide de quinhydrone peut être considérée comme ayant une pression d'hydrogène très faible mais constante, déterminée par cette réaction réciproque:

$$\underset{\text{Hydroquinone}}{C_6H_4O_2H_2} \rightleftharpoons \underset{\text{Quinone}}{C_6H_4O_2} + H_2$$

Cette réaction est représentée par l'équation d'équilibre:

$$[H_2] = k_1 \frac{[C_6H_4O_2H_2]}{[C_6H_4O_2]},$$

où k_1 est une constante, les parenthèses désignant, comme d'habitude, les concentrations molaires des substances mises en jeu. Vu que dans toute solution de quinhydrone pure les concentrations molaires en hydroquinone et quinone seront constamment égales, il résulte que la concentration en hydrogène de la solution, $[H_2]$, sera toujours la même et égale à k_1, quelle que soit

la quantité de quinhydrone dissoute, que la scission de la quinhydrone soit totale ou partielle.

Ce sont ces données qui ont permis à Biilmann de construire son électrode à quinhydrone. Celle-ci se compose d'un vase d'électrodes dans lequel la solution acide aqueuse dont il s'agit de mesurer la concentration en ions hydrogène est installée, après avoir été au préalable secouée en présence d'un peu de quinhydrone à l'état solide; finalement, on plonge dans la liqueur électrolyte une plaque ou un fil de platine bien polis et propres, et l'électrode est prête à servir. Biilmann a démontré que cette électrode si simple et si commode présente, ou du moins ne tarde pas à prendre, un potentiel constant et bien défini, et que, par conséquent, combinée avec une autre électrode (d'hydrogène ou de calomel) à l'aide d'une liqueur intermédiaire convenable, elle pourra servir à la détermination de la concentration en ions hydrogène de la liqueur électrolyte. En mesurant la concentration des ions hydrogène dans un certain nombre de solutions de concentration ionique connue, Biilmann a démontré que l'électrode de quinhydrone est bien utilisable, et, de plus, il a prouvé que cette électrode, en raison de sa pression d'hydrogène extrêmement faible, peut servir dans bien des cas où l'électrode d'hydrogène se montre impropre. Il ne peut donc guère y avoir de doute que ce procédé si simple et si commode ne soit appelé à être généralement admis.

Dans notre Laboratoire Carlsberg, nous avons essayé d'appliquer le procédé à quelques séries d'expériences sur la solubilité des acides aminés dans l'eau et dans des solutions salines de concentration différente en ions hydrogène. Comme méthode de contrôle, nous avons employé la mesure électrométrique usuelle des ions hydrogène. Nous avons pu ainsi constater que les deux méthodes de mesurage donnaient les mêmes résultats dans les solutions contenant pas ou peu de sel, et que, au contraire, il n'en était plus de même pour celles à forte teneur en sel[1]). Vu que ces faits ont une grande importance au point de vue de l'application de l'électrode à quinhydrone, et qu'ils présentent aussi un intérêt considérable sous d'autres rapports, nous en avons fait l'objet d'une recherche quelque peu approfondie.

[1]) Déjà une solution normale de sel accusait une différence de 2 à 3 millivolts, et pour celles plus riches en sel l'écart se montrait beaucoup plus grand.

Un certain nombre de préparations utilisées dans ces recherches ont été obligeamment mises à notre disposition par le Laboratoire chimique de l'Université; nous nous faisons un devoir d'en présenter nos meilleurs remerciements à son directeur, M. le Professeur Biilmann. En même temps nous profitons de cette occasion pour exprimer notre gratitude à M. le Professeur N. Bjerrum, qui a bien voulu discuter avec nous le côté théorique de la question, et dont la connaissance spéciale de ce sujet nous a été de la plus grande utilité.

1°. L'«erreur de sel» de l'électrode à quinhydrone.

Pour rechercher l'«erreur de sel» de l'électrode à quinhydrone, la pile la plus appropriée est celle composée d'une électrode d'hydrogène ordinaire et d'une électrode à quinhydrone, une seule et même liqueur étant utilisée pour l'une et l'autre, soit de l'acide chlorhydrique normal au 1/100 (avec ou sans sel), liqueur qui servira en même temps de liquide intermédiaire entre les deux électrodes.

Une telle pile, dont la construction peut s'exprimer de la façon suivante:

Pt, H_2, HCl 0.01 n — HCl 0.01 n — HCl 0.01 n, quinhydrone, Pt

offre le grand avantage d'être exempt de potentiel de diffusion, de sorte que la tension de la pile ne dépend que du rapport existant entre les pressions d'hydrogène des deux électrodes. Désignons la pression d'hydrogène de l'électrode d'hydrogène par P_{H_2} et la pression d'hydrogène de l'électrode à quinhydrone par P_Q; la tension de la pile, π, sera exprimée par la formule

$$\pi = K\,(\log P_{H_2} \div \log P_Q),$$

où

$$K = \frac{0.0577 + 0.0002\,(t \div 18°)}{2}.$$

Si l'électrode d'hydrogène possède toujours la même pression (soit 1 atmosphère d'hydrogène sec) ou si, à l'aide de la formule de correction bien connue, on rapporte les mesures à cette pression normale, on aura — pourvu que la pression d'hydrogène de l'électrode à quinhydrone, à température constante, reste constamment la même, comme nous l'avons supposé plus haut — une valeur constante de π, quel que soit le caractère du liquide des électrodes. Aussi, les mesures faites par Biilmann, portant

sur des piles de ce genre, ont-elles donné des valeurs constantes de π, abstraction faite des erreurs expérimentales; à 18° et à une pression de 1 atmosphère d'hydrogène sec, Biilmann a trouvé $\pi = 0.7044$ volts. Nous avons aussi, de notre côté, mesuré la tension d'un certain nombre de piles pareilles, et à cet effet nous avons fait usage, comme liqueur électrolyte, principalement d'acide chlorhydrique normal au 1/100, soit sans addition d'aucun sel ou avec addition de chlorure de sodium en proportion plus ou moins forte, jusqu'à une concentration 4-normale en sel.

Les résultats de nos mesures se trouvent réunis dans le Tableau 1. Il ressort de ces chiffres qu'avec une liqueur exempte de sel nous avons trouvé une valeur de π se rapprochant beaucoup de celle indiquée ci-dessus: 0.7044 volts. En même temps, cependant, on voit que π diminue considérablement quand la concentration en sel augmente.

Il faut chercher l'explication de ce résultat dans ce fait, qu'une addition de sel influence différemment les deux composants de la quinhydrone, et, partant, donne lieu à un changement de la pression d'hydrogène de l'électrode à quinhydrone. L'action dont il s'agit ici consiste, pour les deux substances en question, en une augmentation de leur activité; cependant, l'augmentation est de beaucoup plus forte pour l'hydroquinone que pour la quinone.

Aussi longtemps qu'il s'agit de solutions étendues renfermant peu ou pas d'électrolytes, on peut admettre que la masse active ou activité d'un corps dissous est à peu de chose près égale à sa concentration; mais il n'en est plus de même lorsqu'on a affaire à des solutions plus concentrées ou plus riches en électrolytes. Conséquemment, si nous désignons la concentration molaire d'une substance par c et son activité molaire par a, ce n'est que pour les solutions étendues dépourvues d'électrolytes que nous pouvons poser $a = c$; ordinairement nous aurons $a = c \cdot f$, où f, le coefficient d'activité, est un facteur dont la grandeur devra être déterminée par voie expérimentale et qui dépend tant de la substance elle-même que des corps qui agissent sur elle.

Une longue suite de travaux publiés dans ces dernières années par des savants américains, concurremment avec les recherches effectuées par N. Bjerrum et J. N. Brönsted sur l'action des électrolytes forts, sont venus jeter un jour nouveau sur l'action réciproque des ions, laquelle est due aux forces in-

Tableau 1. Mesures de potentiels dans les piles du type Pt, H_2, HCl 0.01 n — HCl 0.01 n — HCl 0.01 n, Quinhydrone, Pt.

Composition de la solution	Valeurs de π trouvées		Moyenne de π π (trouvé)	Terme de correction $0.02885 \times \log \frac{f_{hydroqu.}}{f_{quin.}}$ s	$\pi = \pi_0 \div s$ ($\pi_0 = 0.7048$) π (calculé)
HCl 0.01 n	0.7047	0.7049	0.7048	0.0000	0.7048
	0.7051	0.7049			
	0.7049	0.7046			
	0.7047	0.7048			
	0.7047	0.7048			
	0.7045	0.7048			
	0.7043	0.7048			
	0.7048	0.7048			
	0.7048				
HCl 0.01 n NaCl 0.49 n	0.7040	0.7042	0.7041	0.0013	0.7035
HCl 0.01 n NaCl 0.99 n	0.7024	0.7026	0.7025	0.0028	0.7020
HCl 0.01 n NaCl 1.99 n	0.6989	0.6987	0.6988	0.0058	0.6990
HCl 0.01 n NaCl 2.99 n	0.6961	0.6961	0.6961	0.0089	0.6959
HCl 0.01 n NaCl 3.99 n	0.6933	0.6931	0.6933	0.0125	0.6923
	0.6933	0.6931			
	0.6934	0.6931			
	0.6936	0.6931			

terioniques des particules électriquement chargées. A part cette action spéciale, il faut cependant, ainsi que Brönsted l'a signalé dans une conférence faite à la réunion mentionnée au début de ce mémoire, tenir compte de ce fait que toutes les substances en présence, y compris les électrolytes, exercent une action précipitante sur toutes les autres substances présentes. Si donc il s'agit de l'influence exercée par un électrolyte sur un autre, les choses se compliquent singulièrement en ce sens que, en dehors des forces interioniques, il faut compter avec le pouvoir de précipitation. Dans le cas, au contraire, où il sera question de l'action d'un électrolyte sur des corps non chargés — dans le cas actuel, hydroquinone et quinone —, nous serons en présence d'un

état de choses bien plus simple, puisqu'alors nous n'aurons affaire qu'au pouvoir précipitant. La grandeur de celui-ci peut, comme on le verra plus loin, être déterminée par des essais de dissolution sur les substances pures.

Il est évident que l'équation de l'action des masses qui, formulée de la façon habituelle, contient les concentrations des substances en jeu, devra être modifiée de telle manière que ces concentrations y soient remplacées par les activités desdites substances. Le processus réciproque mentionné ci-dessus, qui détermine la pression d'hydrogène de l'électrode à quinhydrone:

$$\text{Hydroquinone} \rightleftharpoons \text{Quinone} + H_2$$

donne, par conséquent, l'équation d'équilibre suivante:

$$a_{H_2} \cdot a_{quin.} = k_1 \cdot a_{hydroqu.} \qquad (1),$$

où $a_{H_2} = c_{H_2} \cdot f_{H_2}$, $a_{qu.} = c_{qu.} \cdot f_{qu.}$ et $a_{hydroqu.} = c_{hydroqu.} \cdot f_{hydroqu.}$,

et où les différents a, c et f désignent les activités, concentrations et coefficients d'activité des substances expérimentées.

L'équation (1) donne:

$$a_{H_2} = k_1 \frac{a_{hydroqu.}}{a_{quin.}} = k_1 \frac{c_{hydroqu.} \cdot f_{hydropu.}}{c_{quin.} \cdot f_{quin.}}.$$

Dans une solution de quinhydrone on a, cependant, $c_{hqu.} = c_{qu.}$ et, conséquemment, l'activité ou pression d'hydrogène, a_{H_2}, s'obtient par l'équation suivante:

$$a_{H_2} = k_1 \frac{f_{hydroqu.}}{f_{quin.}} \qquad (2)$$

Le mode le plus facile de déterminer les coefficients d'activité dans le cas actuel, est au moyen d'essais de solubilité des substances pures concernées. Si une solution est saturée d'un corps présent en excès à l'état solide, cela revient à dire que l'activité de la substance entrée en dissolution fait équilibre à la tendance de dissolution du corps solide; or, puisque cette tendance, étant une propriété inhérente à la substance solide, est une quantité constante, — désignons-la par A —, il s'ensuit que l'activité de la substance en solution saturée doit, elle aussi, être constante, égale à A, soit que la solution contienne du sel ou non.

Si maintenant nous supposons que le coefficient d'activité d'une solution aqueuse pure, exempte de sel, f_o, puisse être posé égal à 1, nous aurons pour une telle solution saturée:

$$A = a_o = c_o \cdot f_o = c_o,$$

tandis que pour une solution saturée de la substance en question et renfermant du sel de la normalité n, l'équation correspondante pourra s'écrire:

$$A = a_n = c_n \cdot f_n.$$

Ces deux équations donnent:

$$f_n = \frac{c_o}{c_n} \qquad (3)$$

Dans le cas actuel, le coefficient d'activité sera donc égal à une quantité que nous pourrions appeler le coefficient de précipitation par les sels.

Au moyen d'une série d'essais, respectivement avec de l'hydroquinone et de la quinone pures, nous avons déterminé la solubilité de ces substances, à $18° \pm 0°.1$, dans l'acide chlorhydrique normal au 1/100 et dans l'acide chlorhydrique de la même normalité mais dans lequel était dissous du chlorure de sodium en proportion plus ou moins grande; nous n'avons pas employé d'eau pure, puisque la quinhydrone se décompose bien plus vite en milieu neutre qu'en solution légèrement acide. Ces essais de solubilité ont été réalisés à la manière habituelle (rotation à l'étuve pendant quatre heures, succion à travers un bouchon de ouate), la teneur en hydroquinone étant titrée en solution alcaline de bicarbonate, saturée d'acide carbonique, avec de l'iode en excès (dissous en solution d'iodure de potassium), ensuite titration en retour par l'hyposulfite de sodium en présence de l'amidon[1]), tandis que la teneur en quinone était titrée avec un excès d'une solution chlorhydrique de chlorure de titane saturée d'acide carbonique, puis titrage en retour par une solution de ferrisulfate d'ammonium, le rhodanure de potassium servant d'indicateur[2]). Les liqueurs de titrage étaient étalonnées à l'aide de quantités pesées, respectivement, d'hydroquinone et de quinone pures. —

[1]) A. Valeur: Ann. de Chim. et de Phys. [7] **21**, 528 (1900).

[2]) Edm. Knecht und Eva Hibbert: Ber. deutsch. chem. Gesellsch. **43**, 3455 (1910).

Quant au degré de précision de ces méthodes, nous en parlerons dans le chapitre 4.

Les résultats de ces essais de solubilité sont rassemblés dans le Tableau 2 et graphiquement représentés dans la Figure 1, où la molarité de la solution est portée en abscisse, tandis que la normalité globale des chlorures (de sodium et d'hydrogène) est

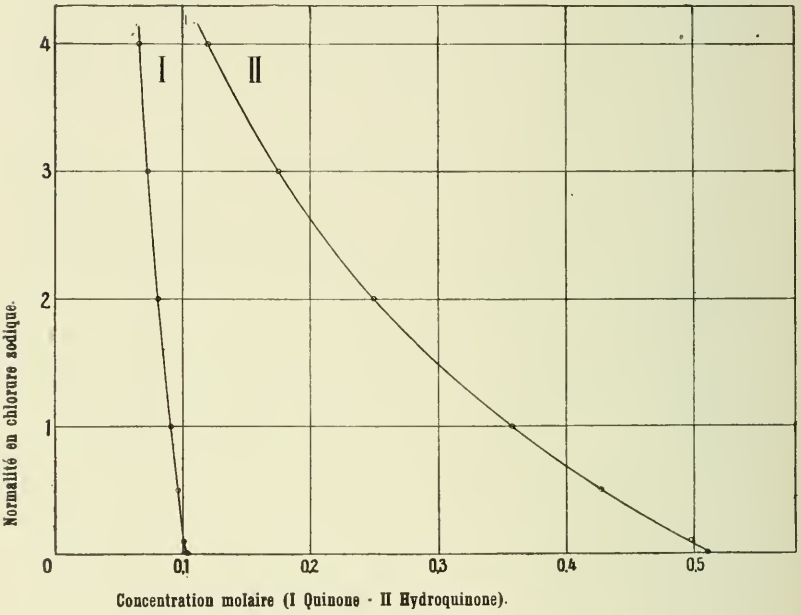

Fig. 1.

employée comme ordonnée. Au moyen de la Figure 1, les solubilités dans l'eau pure inscrites dans le Tableau 2 ont été trouvées par extrapolation graphique. A partir des solubilités trouvées, les coefficients d'activité ont été calculés à l'aide de l'équation (3); ils sont indiqués dans les troisième et cinquième colonnes verticales du Tableau 2 et, graphiquement, dans la Figure 2, où lesdits coefficients sont portés en abscisses, les normalités des chlorures en ordonnées.

A partir des coefficients d'activité calculés, il est maintenant facile de calculer la tension existant dans les piles mentionnées plus haut, formées d'une électrode d'hydrogène ordinaire et d'une électrode de quinhydrone, avec une solution de chlorure de sodium quelconque comme liqueur électrolyte.

Employons la formule de Biilmann:

$$\pi = K \, (\log P_{H_2} \div \log P_Q),$$

avec cette différence toutefois que, alors que Biilmann pose P_Q, la pression d'hydrogène de l'électrode à quinhydrone, égal à $k_1 \frac{c_{hydroqu.}}{c_{quin.}}$, ce qui revient à dire égal à k_1, nous posons

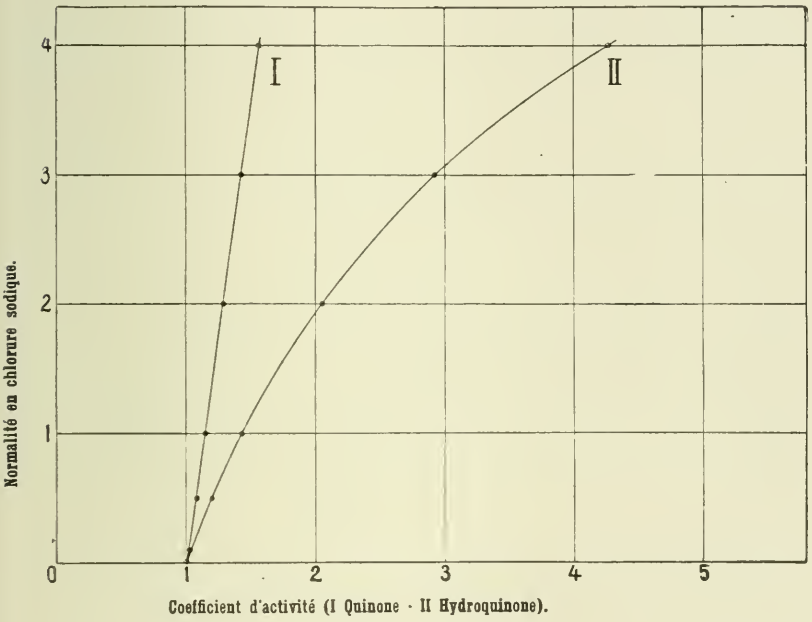

Fig. 2.

$$P_Q = a_{H_2} = k_1 \frac{c_{hydroqu.} \cdot f_{hydroqu.}}{c_{quin.} \cdot f_{quin.}} = k_1 \frac{f_{hydroqu.}}{f_{quin.}}.$$

Nous aurons donc

$$\pi = K \left(\log P_{H_2} \div \log k_1 \div \log \frac{f_{hydroqu.}}{f_{quin.}} \right) \qquad (4)$$

Pour la normalité de sel 0, on a $f_{hydroqu.} = f_{quin.} = 1$, donc

$$\pi_0 = K \, (\log P_{H_2} \div \log k_1)$$

$$\pi = \pi_0 \div K \log \frac{f_{hydroqu.}}{f_{quin.}}, \qquad (5)$$

où K (pour t = 18°) est égal à $^1/_2 \cdot 0.0577 = 0.02885$.

Tableau 2. Solubilité de la quinone et de l'hydroquinone dans l'acide chlorhydrique 0.01 n sans et avec chlorure de sodium. (Mol. par litre de solùtion).

Normalité de HCl + NaCl.	Molarité de la solution saturée de Quinone A	$\left(\frac{A_o}{A_n}\right)$ $f_{quinone}$	Molarité de la solution saturée d'Hydroquinone B	$\left(\frac{B_o}{B_n}\right)$ $f_{hydroquinone}$	$\frac{f_{hydroqu.}}{f_{quin.}}$	$0.02885 \times \log \frac{f_{hydroqu.}}{f_{quin.}}$
0	0.10390 (extrap.)		0.51200 (extrap.)			
0.01 n	0.10368	1.002	0.51030	1.003	1.001	0.0000
0.1 -	0.10094	1.029	0.49762	1.029	1.000	0.0000
0.5 -	0.09608	1.081	0.42704	1.199	1.109	0.0013
1.0 -	0.09049	1.148	0.35794	1.430	1.246	0.0028
2.0 -	0.08036	1.293	0.24956	2.052	1.587	0.0058
3.0 -	0.07262	1.431	0.17523	2.922	2.042	0.0089
4.0 -	0.06614	1.571	0.11994	4.269	2.717	0.0125

Les valeurs calculées pour le terme de correction $K \log \frac{f_{hydroqu.}}{f_{quin.}}$ se trouvent indiquées dans le Tableau 2, dernière colonne verticale, ainsi que dans le Tableau 1, quatrième colonne verticale, tandis que les valeurs de π calculées à l'aide de l'équation (5) sont inscrites dans la cinquième colonne du Tableau 1.

En comparant les valeurs ainsi calculées de π avec celles trouvées par mesurage de la force électromotrice, on verra que, si la différence entre les valeurs trouvées et celles calculées n'est pas très considérable, elle est sans doute, pour ce qui concerne la solution la plus riche en sel, un peu plus grande que l'erreur expérimentale dont il faut tenir compte ici. Ceci tient probablement à ce que le degré de précision avec lequel ont été déterminés les coefficients d'activité — et, conséquemment, le rapport entre eux — n'est pas tout à fait incontestable, en raison du procédé employé à cet effet. C'est que, même pour les concentrations très faibles en quinone et hydroquinone dont il est question dans les solutions de quinhydrone, nous avons compté avec les coefficients d'activité trouvés pour des solutions saturées, bien plus fortes, des deux substances. D'un autre côté, il ne peut guère y avoir de doute que l'«erreur de sel» affectant l'électrode à quinhydrone ne doive s'expliquer de la manière que nous venons d'esquisser.

2°. Electrodes Quinone-Hydroquinone où le rapport entre les deux composants est arbitrairement choisi.

La formule établie par Biilmann pour une pile composée d'une électrode d'hydrogène et d'une électrode à quinhydrone avec un même liquide électrolyte:

$$\pi = K (\log P_{H_2} \div \log P_Q)$$

garde évidemment sa validité lorsque l'électrode quinhydrone est remplacée par une électrode quinone-hydroquinone, dans le liquide électrolyte de laquelle ces deux corps ne sont pas en rapport équimolaire l'un à l'autre, pourvu que, si nous désignons par P_Q la pression d'hydrogène d'une telle électrode, nous posions:

$$P_Q = a_{H_2} = k_1 \frac{c_{hydroqu.} \cdot f_{hydroqu.}}{c_{quin.} \cdot f_{quin.}},$$

où cependant $c_{hydroqu.} \gtrless c_{quin.}$

Le potentiel d'une pareille pile peut être calculé de la même manière que le potentiel des piles dont il a été question dans le chapitre précédent. En effet, on a:

$$\pi = K \left(\log P_{H_2} \div \log k_1 \div \log \frac{c_{hydroqu.}}{c_{quin.}} \div \log \frac{f_{hydroqu.}}{f_{quin.}} \right) \qquad (6)$$

De plus, nous avons vu dans le même chapitre (page 9) que le potentiel, π_0, d'un élement composé d'une électrode d'hydrogène et d'une électrode à quinhydrone pure avec un liquide électrolyte exempt de sel peut s'exprimer ainsi:

$$\pi_0 = K (\log P_{H_2} \div \log k_1) \qquad (7)$$

En retranchant l'équation (7) de (6), on aura:

$$\pi = \pi_0 \div K \log \frac{c_{hydroqu.}}{c_{quin.}} \div K \log \frac{f_{hydroqu.}}{f_{quin.}} \qquad (8)$$

Ainsi donc, pour pouvoir calculer π, il faut connaître non seulement le rapport entre les coefficients d'activité, mais encore le rapport entre les concentrations en hydroquinone et en quinone. Dans un mélange arbitrairement choisi de quinone et d'hydroquinone, il y aura cependant toujours une quantité, plus ou moins grande selon les circonstances, de quinhydrone non dissociée. Cette proportion de quinhydrone, dont nous désignerons la concentration par u, n'entre pas dans le rapport $\frac{c_{hydroqu.}}{c_{Quin.}}$ et, par conséquent, pour la calculation de π il est nécessaire de connaître non seulement la concentration totale en quinone, que nous appellerons A, et la concentration totale en hydroquinone, B, mais aussi u; nous aurons alors:

$$\frac{c_{hydroqu.}}{c_{quin.}} = \frac{B \div u}{A \div u} \qquad (9)$$

La grandeur de u dépend du degré de dissociation de la quinhydrone, qui peut être déterminée au moyen de l'équation d'équilibre suivante:

$$a_{quin.} \cdot a_{hydroqu.} = k_2 \cdot a_{quinhydr.}$$
$$c_{quin.} \cdot f_{quin.} \cdot c_{hydroqu.} \cdot f_{hydroqu.} = k_2 \cdot u \cdot f_{quinhydr.}$$

Si maintenant nous y substituons, à $c_{quin.}$, $A \div u$, et à $c_{hydroqu.}$, $B \div u$,

et si nous posons

$$\frac{f_{quinhydr.}}{f_{quin.} \cdot f_{hydroqu.}} = F,$$

nous aurons:

$$(A \div u)\ (B \div u) = k_2 \cdot u \cdot F$$
$$AB \div u\,(A + B) = k_2 \cdot u \cdot F \div u^2 \qquad (10)$$

équation qui s'applique à toutes les solutions qui contiennent aussi bien de la quinone que de l'hydroquinone. Par contre, elle ne permet évidemment pas de calculer u, même si A et B sont connus; car, à côté de u, l'équation renferme deux autres inconnues: k_2 et F. Pourtant, si pour une solution d'électrolyte déterminée on connaît k_2 et F ou seulement le produit $k_2 \cdot F$, on peut calculer u à l'aide de l'équation (10) pour des concentrations arbitrairement choisies et connues de quinone et d'hydroquinone (A et B) dans ladite solution.

Afin de trouver la grandeur du produit $k_2 \cdot F$ pour une solution d'électrolyte donnée, nous avons procédé de la manière suivante:

Tout d'abord, par une série d'essais, nous avons établi la solubilité de la quinhydrone dans la solution d'électrolyte en question préalablement additionnée, dans chacun des essais, de l'un des composants de la quinhydrone, par exemple l'hydroquinone, en proportion différente. Dans ces conditions, il est légitime d'admettre que les coefficients d'activité, et partant F aussi, sont constants dans tous ces essais, puisque la même solution d'électrolyte à servi à tous. — En outre, nous avons employé dans tous les essais un excès de quinhydrone, de sorte que $a_{quinhydr.}$ est constant; et comme $f_{quinhydr.}$ est constant, u doit aussi l'être. Si u et F sont constants, le côté droit de l'équation (10) le sera également, et cette équation sera celle d'une ligne droite. Par conséquent, si par une analyse aussi exacte que possible, on établit les valeurs de A et B dans les différents essais d'une pareille série, et si l'on porte en abscisses le produit des valeurs trouvées pour A et B, en ordonnées la somme de ces valeurs, les résultats expérimentaux de toute la série devront pouvoir être exprimés par une ligne droite, dont la direction détermine la valeur de u; car

$$u = \frac{A_1 \cdot B_1 \div A_2 \cdot B_2}{(A_1 + B_1) \div (A_2 + B_2)} \qquad (11)$$

Une fois que u aura été déterminé de cette manière, on pourra calculer $k_2 \cdot F$ pour chaque essai en particulier à l'aide de l'équation (10).

Ainsi que Biilmann l'a fait dans les essais de ce genre effectués par lui sur la solubilité dans l'acide sulfurique normal au 1/100, on peut évidemment substituer dans la formule (11) les

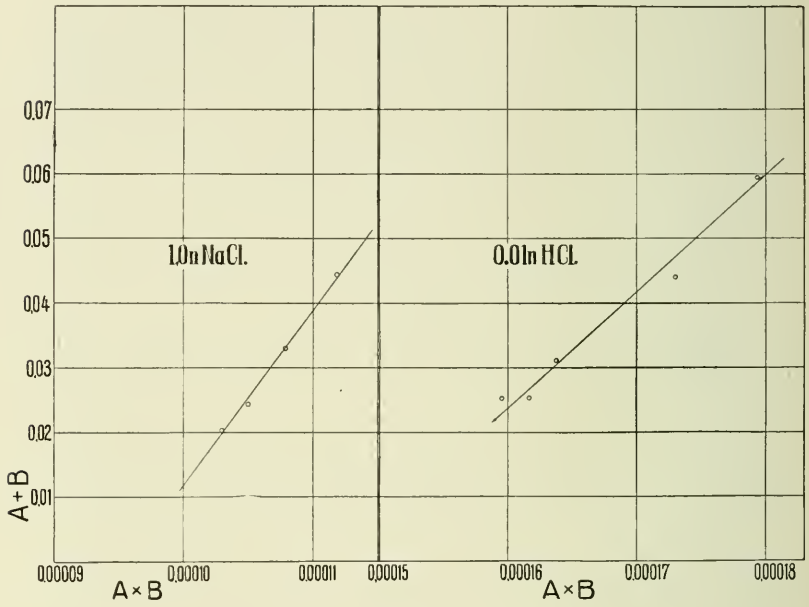

Fig. 3.

valeurs directement trouvées pour A et B; vu cependant qu'il s'agit ici de différences extrêmement minimes — surtout dans les cas des solutions riches en sel —, il s'ensuit que de légères erreurs faites dans les déterminations pourront donner lieu à des variations considérables dans u. C'est pourquoi nous avons préféré la représentation graphique esquissée plus haut, qui donne un aperçu exact de tous nos résultats expérimentaux, et qui permet d'égaliser les erreurs.

Ces expériences nous ont, du reste, causé pas mal de difficultés et, malgré tous les soins apportés à ce travail, nous ne sommes pas parvenus à réaliser, entre les résultats obtenus dans chaque série d'essais en particulier, une concordance aussi bonne que celle obtenue par Biilmann dans ses expériences. Nous

pensons qu'il faut en chercher la raison soit dans l'incertitude où nous étions à l'égard de l'influence exercée sur la grandeur des coefficients d'activité par les quantités présentes de quinone et d'hydroquinone (voir la fin du chapitre précédent), soit, et surtout, dans un fait qui sera mentionné en détail au 4ième chapitre, à savoir que les différentes préparations de quinhydrone que nous avions entre les mains se sont trouvées n'avoir pas la même solubilité. Dans nos séries d'essais finales, nous avons cependant employé un seul et même échantillon de quinhydrone, preparé et lavé très soigneusement, et nous avons été ainsi conduits aux résultats exposés ci-dessous, que nous ne regardons pas comme tout à fait sûrs, il est vrai, mais pourtant comme parfaitement utilisables pour le but que nous nous sommes proposé. — Au reste, l'un de nous, M. K. Linderstrom-Lang, est en train de soumettre à une vérification plus approfondie les sources d'erreur en question.

Dans le Tableau 3 se trouvent rassemblés les résultats de trois de ces séries d'essais; les deux premières séries, où les concentrations d'électrolytes sont, respectivement, normale au 1/100 et normale, sont en outre représentées graphiquement par la Figure 3, où $A \cdot B$ est porté en abscisse et $A + B$ en ordonnée. En tenant un compte égal de chaque résultat dans les différentes séries, nous avons ensuite tracé la ligne droite qui détermine la grandeur de u. Par substitution dans l'équation (10) des valeurs de u ainsi trouvées (Tableau 3, cinquième colonne verticale), $k_2 \cdot F$ a alors été calculé pour chaque essai de cette série. Le Tableau 3, dernière colonne verticale, où se trouvent inscrites les valeurs de $k_2 \cdot F$ ainsi calculées, montre que ce produit paraît être le même pour la solution d'électrolyte normale que pour celle normale au 1/100, et ce résultat nous a conduits à la conclusion — correcte par première approximation au moins —, que la grandeur du produit $k_2 \cdot F$ est indépendante de la concentration en électrolyte de la solution et égale à la moyenne des valeurs trouvées: 0.263. Il eût été souhaitable que la justesse de cette conclusion eût pu être confirmée par la troisième série d'essais rapportée dans le Tableau 3, essais où la concentration électrolytique est 3-normale; mais les résultats auxquels a conduit cette série, où u est fort minime et de ce fait difficile à déterminer, ne sont pas suffisamment précis pour nous permettre

Tableau 3. Détermination de u et de $k_2 \times F$.

Dans tous ces essais on a employé 200 cm³ de la solution d'électrolyte, dans laquelle étaient dissoute les doses d'hydroquinone indiquées dans la deuxième colonne verticale. Les solutions furent secouées quatre heures, dans l'étuve à $18^0 \pm 0.^0 1$, avec un abondant excès de quinhydrone.

Solution d'électrolyte	Dose d'hydroquinone g.	Molarité trouvée par analyse, de la solution saturée (mol. par litre de solution) Quinone A	Hydroquinone B	u	$k_2 \times F$
HCl 0.01 n	0	0.01276	0.01267	0.000557 (trouvé graphiquement)	0.265
	0	0.01264	0.01263		0.262
	0.4	0.00672	0.02438		0.263
	0.8	0.00437	0.03963		0.267
	1.2	0.00319	0.05624		0.263
HCl 0.01 n NaCl · 0.99	0	0.01016	0.01014	0.000365 (trouvé graphiquement)	0.262
	0.27	0.00563	0.01864		0.264
	0.54	0.00368	0.02934		0.263
	0.81	0.00268	0.04174		0.262
HCl 0.01 n NaCl · 2.99	0	0.00648	0.00648	0.000155 (valeur moyenne de $k_2 \times F = 0.263$)	0.258
	0.14	0.00385	0.01109		0.261
	0.28	0.00263	0.01692		0.268
	0.42	0.00192	0.02345		0.265

Moyenne des neuf premières valeurs: 0.263

une détermination sûre de u[1]). Dans ces conditions, nous avons cru justifiable d'assigner à u une valeur moyenne telle que, pour les quatres essais constituant la série, $k_2 \cdot F$ se rapproche de la valeur 0.263 d'aussi près que possible: il en sera ainsi — comme le montre le tableau —, si l'on donne à u la valeur 0.000155.

S'il est juste d'admettre que — quelle que soit la concentration électrolytique — le produit $k_2 \cdot F$ soit constant et égal à 0.263, il s'ensuivra que $k_2 = 0.263$ et $F = 1$, puisque la valeur 0.263 devra être vraie pour les solutions exemptes d'électrolytes,

[1]) En traçant une ligne par les deux points correspondant aux deux premièrs essais de la série, on arrive à une valeur évidemment trop élevée de u, tandis que, si la ligne est tracée par les deux derniers points, u devient trop bas.

Tableau 4. Calcul de u pour $k_2 \times F = 0.263$.

Solutions saturées de quinhydrone. Les solutions d'électrolytes ont été secouées durant quatre heures, dans l'étuve à $18^0 \pm 0^0.1$, avec un abondant excès de quinhydrone.

Solution d'électrolyte	Molarité, trouvée par analyse, de la solution saturée (mol. par litre de solution)		u	Degré de dissociation de la quinhydrone $\alpha = \frac{A \div u}{A} = \frac{B \div u}{B}$
	Quinone A	Hydroquinone B		
HCl 0.01 n	0.01276 0.01264	0.01267 0.01263	0.000558 (0.000557)	0.956
HCl 0.01 n NaCl 0.09 n	0.01233	0.01247	0.000535	0.957
HCl 0.01 n NaCl 0.49 n	0.01141	0.01135	0.000454	0.960
HCl 0.01 n NaCl 0.99 n	0.01016	0.01014	0.000364 (0.000365)	0.964
HCl 0.01 n NaCl 1.99 n	0.00800	0.00800	0.000230	0.971
HCl 0.01 n NaCl 2.99 n	0.00648	0.00648	0.000152 (0.000155)	0.976
HCl 0.01 n NaCl 3.99 n	0.00517	0.00513	0.000097	0.981

ou les coefficients d'activité doivent être considérés comme égaux à l'unité. Il n'y a rien qui soit de prime abord contraire à la supposition que $F = 1$ et, par conséquent, $f_{quin.} \cdot f_{hydroqu.} = f_{quinhydr.}$ et les expériences relatées ci-dessus paraissent rendre vraisemblable que cette supposition est à tel point exacte que nous pouvons en faire usage sans commettre d'erreur appréciable.

En vue d'éclaircir ultérieurement les questions relatives à la solubilité de la quinhydrone, nous avons rassemblé dans le Tableau 4 les résultats de quelques essais faits à cet égard avec de la quinhydrone pure que, sans y ajouter aucun des composants, nous fîmes dissoudre dans un certain nombre de solutions de sel marin légèrement acides. Au moyen des concentrations trouvées dans les solutions saturées, nous avons calculé u (pour $k_2 \cdot F = 0.263$) ainsi que le degré de dissociation de la quin-

Tableau 5. Piles composées d'une électrode d'hydrogène et d'une électrode quinone et hydroquinone, le Rapport entre les Concentrations en quinone et en hydroquinone étant arbitrairement choisi et la solution électrolyte étant la même dans la pile entière.

Solution électrolyte	Composition de la solution (Mol. par litre de solution)		u	$0.02885 \times \log \frac{B \div u}{A \div u}$	$0.02885 \times \log \frac{f_{hydroqu.}}{f_{quin.}}$	π (calculé)	π (mesuré)
	Quinone A	Hydroquinone B				Volt	Volt
HCl 0.01 n	0.001675	0.004743	0.000029	+ 0.0132	0.0000	0.6916	0.6919 0.6919
HCl 0.01 n	0.004598	0.007528	0.000126	+ 0.0063	»	0.6985	0.6988 0.6992
HCl 0.01 n	0.009196	0.003764	0.000125	÷ 0.0114	»	0.7162	0.7163 0.7163
HCl 0.01 n	0.005025	0.001581	0.000029	÷ 0.0146	»	0.7194	0.7194 0.7194
HCl 0.01 n	0.003146	0.007148	0.000082	+ 0.0105	0.0058	0.6885	0.6888 0.6888
NaCl 1.99 n	0.006292	0.003574	0.000082	÷ 0.0072	»	0.7062	0.7064 0.7064
HCl 0.01 n NaCl 2.39 n	0.005025	0.001581	0.000029	÷ 0.0146	0.0070	0.7124	0.7130 0.7130
HCl 0.01 n	0.003112	0.003560	0.000064	+ 0.0074	0.0125	0.6849	0.6864 0.6864
NaCl 3.99 n	0.006224	0.002780	0.000064	÷ 0.0103	»	0.7026	0.7035 0.7037

hydrone; aux valeurs calculées de u, nous avons joint entre parenthèses celles inscrites au Tableau 3.

Revenons maintenant au sujet principal de ce chapitre, le calcul de π pour une pile composée d'une électrode d'hydrogène et d'une électrode quinone-hydroquinone, dont le rapport entre les deux composants est quelconque, et la solution d'électrolyte étant la même dans l'élément entier. Au début de ce chapitre, il a été montré que le potentiel d'une telle pile peut être calculé à l'aide de l'équation (8):

$$\pi = \pi_0 \div K \log \frac{c_{hydroqu.}}{c_{quin.}} \div K \log \frac{f_{hydroqu.}}{f_{quin.}},$$

où $\pi_0 = 0.7048$ volts et $K = 0.02885$ à 18°, et où $c_{quin.} = A \div u$ et $c_{hydroqu.} = B \div u$.

Afin de démontrer l'exactitude de cette équation, nous avons effectué une série d'essais, dont les résultats sont consignés dans le Tableau 5. Des doses pesées de quinone et d'hydroquinone furent dissoutes dans des solutions d'électrolyte similaires, puis mélangées dans des proportions connues, de façon à produire un mélange de composition connue (Tableau 5, deuxième et troisième colonnes verticales). Cela fait, on a calculé u à l'aide de l'équation (10), en supposant $k_2 \cdot F = 0.263$. Un calcul simple donna alors le terme de correction

$$0.02885 \cdot \log \frac{B \div u}{A \div u},$$

tandis que le terme de correction $0.02885 \cdot \log \frac{f_{hydroqu.}}{f_{quin.}}$ se trouvait déjà indiqué dans le Tableau 2.

Une comparaison entre les valeurs de π calculées d'après l'équation (8) et celles trouvées en mesurant la force électromotrice (Tableau 5, deux dernières colonnes), montre une bonne concordance; toutefois, ici encore, les deux mélanges les plus riches en sel donnent au calcul une valeur de π un peu moindre que celle obtenue par la mesure du potentiel. (Comp. Tableau 1 et p. 11).

3°. Electrodes à quinhydrone sans «erreur de sel».

Une électrode à quinhydrone sans «erreur de sel» peut être construite par saturation du liquide électrolyte avec de la quinhydrone aussi bien qu'avec l'un des composants de celle-ci.

En effet, les deux équations fondamentales et de valeur générale:

$$\text{Hydroquinone} \rightleftharpoons \text{Quinone} + H_2$$

et

$$\text{Quinhydrone} \rightleftharpoons \text{Quinone} + \text{Hydroquinone}$$

donnent les équations suivantes, indiquées déjà dans ce qui précède:

$$a_{H_2} \cdot a_{quin.} = k_1 \cdot a_{hydroqu.}$$

et

$$a_{quin.} \cdot a_{hydroqu.} = k_2 \cdot a_{quinhydr.},$$

Or, nous avons vu que, dans une solution saturée de quinhydrone et, par exemple, d'hydroquinone, tant $a_{quinhydr.}$ que $a_{hydroqu.}$ sont constants. Par conséquent, dans les deux équations de variables il n'y en aura que deux, savoir a_{H_2} et, dans le cas actuel, $a_{quin.}$, et, dès lors, ces deux quantités se trouvent parfaitement définies par les deux équations. En dégageant $a_{quin.}$ de la seconde équation, on aura au moyen de la première:

$$a_{H_2} = \frac{k_1 \cdot a^2_{hydroqu.}}{k_2 \cdot a_{quinhydr.}} = K.$$

Ainsi donc, la pression de l'hydrogène dans une pareille électrode est constante, et une pile formée de cette électrode et d'une électrode d'hydrogène, la solution électrolyte étant la même dans la pile entière, devra montrer un potentiel constant, indépendant de la composition de la solution d'électrolyte. On peut évidemment calculer ce potentiel constant de la même manière que ceux des piles dont il a été question dans le chapitre précédent, à savoir au moyen de l'équation (8):

$$\pi = 0.7048 + 0.02885 \log \frac{c_{hydroqu.}}{c_{quin.}} + 0.02885 \log \frac{f_{hydroqu.}}{f_{quin.}},$$

pourvu que les concentrations en quinone et en hydroquinone, A et B, des solutions saturées soient déjà connues ou puissent être déterminées par des analyses. Cependant, on se heurte ici à une difficulté: c'est qu'une solution saturée de quinhydrone et de l'un des composants de celle-ci ne contient qu'une proportion infime de l'autre composant et que, par suite, la concentration de ce dernier ne peut pas être déterminée de façon certaine; de plus, comme précisément le terme de correction qui dans le cas actuel est le plus important dans l'équation (8) contient le rapport entre les concentrations de l'hydroquinone et de la quinone, il en résulte que la détermination de ce terme devient incertaine.

Tableau 6. Electrodes Quinhydrone sans «Erreur de sel».

Solution électrolyte	La Solution était saturée de	Molarité, trouvée analytiquement, de la solution saturée (Mol. par litre de solution)		u	$0.02885 \times \log \frac{B \div u}{A \div u}$	$0.02885 \times \log \frac{f_{hydroqu}}{f_{quin.}}$	π (calculé)	π (mesuré)
		Quinone A	Hydroquinone B				Volt	Volt
HCl 0.01 n	Quinhydrone et Quinone	0.10353	0.002383	0.000557	÷ 0.0505	0.0000	0.7553	0.7550
								0.7548
HCl 0.01 n, NaCl 0.49 n		0.09567	0.001829	0.000454	÷ 0.0531	0.0013	0.7566	0.7550
								0.7550
HCl 0.01 n, NaCl 1.99 n		0.08000	0.001171	0.000230	÷ 0.0556	0.0058	0.7546	0.7548
								0.7548
HCl 0.01 n, NaCl 3.99 n		0.06591	0.000658	0.000097	÷ 0.0597	0.0125	0.7520	0.7544
								0.7546
						Moyenne ...	0.7546	0.7548
HCl 0.01 n	Quinhydrone et Hydroquinone	0.000979	0.51072	0.000557	+ 0.0889	0.0000	0.6159	0.6187
								0.6191
HCl 0.01 n, NaCl 0.49 n		0.000835	0.42699	0.000454	+ 0.0880	0.0013	0.6155	0.6191
								0.6195
HCl 0.01 n		0.000570	0.24860	0.000230	+ 0.0826	0.0058	0.6164	0.6193
								0.6193
NaCl 1.99 n		0.000583	0.24981	»	+ 0.0822	0.0058	0.6168	—
HCl 0.01 n, NaCl 3.99 n		0.000357	0.12125	0.000097	+ 0.0770	0.0125	0.6153	0.6191
								0.6191
						Moyenne ...	0.6160	0.6191

Cette remarque s'applique surtout aux solutions saturées de quinhydrone et d'hydroquinone, parce que, du fait de la grande solubilité de l'hydroquinone, le rapport susmentionné est ici particulièrement élevé. Nous avons pourtant pensé que, malgré ces difficultés, il y aurait intérêt à préparer et à examiner avec le plus grand soin possible quelques solutions de ce genre, afin d'évaluer par des mesures de potentiel la tension des piles en question et de démontrer son indépendance de la concentration des sels de la liqueur électrolyte et, d'autre part, afin d'établir jusqu'à quel point la valeur de π calculée à partir des déterminations analytiques se rapproche de celle trouvée au moyen des mesures de la force électromotrice.

Le Tableau 6 résume quelques essais faits à ce sujet. Une solution électrolytique ayant la composition indiquée dans la première colonne verticale de ce tableau fut d'abord secouée avec un excès, respectivement, de quinone ou d'hydroquinone, puis additionnée d'un abondant excès de quinhydrone pulvérisée, après quoi le tout fut soumis pendant plusieurs heures à la rotation dans l'étuve à $18° \pm 0°.1$. La solution saturée fut sucée à travers un petit bouchon d'ouate, et la liqueur filtrée parfaitement limpide fut employée en partie pour des mesures de potentiel, en partie pour la détermination analytique de la concentration de la quinone et de l'hydroquinone (A et B). Les valeurs assignées à u étaient empruntées au Tableau 4.

L'inspection de la dernière colonne du Tableau 6 fait voir que les électrodes à quinhydrone telles que celles dont il est question ici ont un potentiel constant, indépendant de la concentration saline, et une comparaison des deux dernières colonnes verticales du tableau montre qu'en tout cas l'ordre de grandeur des potentiels calculés est conforme à la vérité; pour ce qui concerne l'électrode à quinhydrone et à quinone, la concordance entre la moyenne calculée et la moyenne trouvée est même meilleure qu'on ne pourrait s'y attendre en tenant compte des erreurs analytiques inévitables.

4°. Du Degré de Précision des Méthodes de Titrage employées et de la Pureté des Substances utilisées.

Les méthodes de titrage dont nous nous sommes servis pour l'évaluation, respectivement de la quinone et de l'hydroquinone, ont été expliquées déjà dans le premier chapitre. Nous allons

ici rendre brièvement compte des essais de contrôle effectués dans l'intention de vérifier si ces méthodes conviennent bien au but que nous nous sommes proposé.

a. Titrage de la Quinone.

La teneur en quinone fut titrée par addition d'un excès d'une solution de chlorure de titane chlorhydrique saturée d'acide carbonique, puis titration en retour au moyen d'une solution de ferrisulfate d'ammonium en présence du rhodanure de potassium. La solution de ferrisulfate d'ammonium était étalonnée à l'aide de doses de quinone exactement pesées.

Dans le but de vérifier la précision de la méthode en présence de sels, d'hydroquinone etc., nous avons effectué une série d'essais, dans lesquels le titre d'une même solution de ferrisulfate fut établi dans des conditions différentes. Les résultats de ces essais se trouvent rassemblés dans le Tableau 7, qui n'aura pas besoin d'explication.

Il ressort de ce tableau que le résultat de l'étalonnage est sensiblement identique, même avec des variations assez considérables dans les quantités de quinone et de liqueur dissolvante ainsi que dans celle d'hydroquinone présente. Le titre est aussi le même dans les cas d'une solution exempte de sel que dans ceux où elle en contient, et l'emploi comme étalon, de quinhydrone ou bien d'une quelconque des préparations de quinone a notre disposition, s'est trouvé n'avoir pas d'influence non plus. Par contre, la solution de quinone employée doit être de préparation assez récente; autrement, comme le montrent les trois derniers essais du Tableau, il y aura décomposition de quantités appréciables de quinone, ce qui se traduira par un titre trop élevé. Abstraction faite de ces trois derniers essais, l'écart d'avec la moyenne, sauf pour une seule détermination, n'atteint pas $^1/_2$ pour cent; le plus souvent, il est même très inférieure à cette limite.

Quant à la question de savoir quelles sont les substances auxquelles donne naissance la décomposition de la quinone, nous ne l'avons pas fait l'objet de recherches approfondies. On sait cependant que les solutions aqueuses de quinone, laissées en repos pendant un temps suffisamment long, peuvent faire tomber de la quinhydrone, et, d'accord avec cette observation, il n'est pas difficile, à l'aide de la méthode de titrage mentionnée plus

Tableau 7. Essais de Contrôle relatifs au Titrage de la Quinone.

Quinone employée	Quantité de quinone employée mg.	Teneur en hydroquinone de la solution mg.	D'autres substances dans la solution	Volume de la solution avant titrage cm^3	Durée de repos de la solution avant titrage en heures	Quantité de *Quinone* en *mg* correspondant à 1 cm^3 de solution de ferrisulfate d'ammonium
Prép. p. nous-mêmes Recrist. dans l'eau	30	0	60 cm^3 HCl 0.01 n	60	2—3	5.512
—	30	100	—	60	—	5.461
—	60	0	—	60	—	5.495
—	60	80	—	60	—	5.490
—	90	0	—	60	—	5.496
—	90	60	—	60	—	5.491
—	120	0	—	60	—	5.518
—	120	20	—	60	—	5.514
—	50	0	10 cm^3 HCl 0.01 n	10	—	5.512
—	50	0	40 —	40	—	5.518
—	50	55	40 —	40	—	5.500
—	50	165	40 —	40	—	5.506
—	100	0	20 —	20	—	5.512
—	100	0	40 —	40	—	5.515
—	150	0	40 —	40	—	5.494
—	150	55	40 —	40	—	5.512
Labor. chimique de l'Université	66	0	20 cm^3 HCl 0.01 n	20	—	5.504
—	66	0	60 —	60	—	5.510
—	66	0	(20 — / 40 cm^3 NaCl 3 n)	60	—	5.510
—	33	0	10 cm^3 HCl 0.01 n	10	—	5.500
—	33	0	50 —	50	—	5.502
—	33	0	(10 — / 40 cm^3 NaCl 3 n)	50	—	5.510
—	66	0	20 cm^3 HCl 0.01 n	20	6—7	5.530
Prép. p. nous-mêmes Recr. dans l'eau	66	0	25 cm^3 HCl 0.01 n	25	3	5.505
— après dessiccation sur $CaCl_2$	66	0	25 —	25	3	5.488
Quinhydrone	55	56	50 —	50	3	5.508
Prép. p. nous-mêmes Recr. dans l'eau	62	0	25 cm^3 HCl 0.01 n	25	$1-1^1/_2$	5.534
—	62	0	25 cm^3 HCl 0.1 n	25	—	5.490
Labor. chim. de l'Université	62	0	—	25	—	5.506
Prép. p. nous-mêmes Recr. dans l'eau	62	0	25 cm^3 HCl 0.01 n	25	21—22	(5.575)
—	62	0	25 cm^3 HCl 0.1 n	25	—	(5.565)
Labor. chim. de l'Université	62	0	—	25	—	(5.574)
					Moyenne...	5.505

Tableau 8. Proportion d'Hydroquinone dans des Solutions de Quinone abandonnées à la température du laboratoire pendant des temps différents.

On a employé dans chaque essai 25 cm³ de solution de quinone, contenant env. 63 mg de quinone.

Quinone employée	Substances contenues dans la solution	Durée de repos avant titrage en heures	Hydroquinone formée pour cent de la proportion correspondant à l'entière quantité de quinone	Remarques
Préparée par nous-mêmes recr. dans l'eau	25 cm³ HCl 0.01 n	1	0.54 %	Après dix heures de repos, on a remarqué un changement de couleur; mais seulement au bout de vingt-deux heures la solution est devenue nettement d'un jaune tirant sur le rouge.
—	—	3	0.54 -	
—	—	10	0.63 -	
—	—	22	1.17 -	
—	25 cm³ HCl 0.1 n	1	0.18 -	Tout à fait comme les quatre solutions précédentes.
—	—	3	0.36 -	
—	—	10	0.36 -	
—	—	22	0.59 -	
—	25 cm³ H_2SO_4 0.1 n	1	1.26 -	Additionnée de bicarbonate de sodium, la solution a pris de suite une teinte rougeâtre.
—	—	3	3.11 -	Avant l'addition de bicarbonate de sodium, la solution était d'un jaune rougeâtre.
—	—	10	4.73 -	Plus nettement jaune rougeâtre.
—	—	22	5.94 -	Coloration nettement rougeâtre.
Labor. chim. de l'Université	—	1	1.08 -	Tout à fait comme les quatre solutions précédentes.
—	—	3	3.56 -	
—	—	10	4.91 -	
—	—	22	6.12 -	

loin de l'hydroquinone, de déceler dans les solutions acides de quinone, même après un repos relativement court, la présence d'hydroquinone ou en tout cas de corps dont le titrage fait croire à la présence de ce corps. Le Tableau (8) donne les résultats de quelques essais réalisés en vue d'éclaircir ce fait.

Ce tableau, qui n'aura pas besoin d'autre explication, fait voir que, comme on devait s'y attendre, la quantité d'hydroquinone formée va en augmentant avec le temps. En outre, on

voit que la vitesse de décomposition, tout en étant sensiblement la même dans l'acide chlorhydrique normal au 1/100 que dans ce même acide décime[1]), est beaucoup plus grande dans l'acide sulfurique décime que dans l'acide chlorhydrique. La différence, qui se reconnaît facilement déjà au changement de coloration subi par la solution sulfurique, est si frappante, qu'elle paraît rendre désirable des recherches ultérieures sur l'influence exercée par les divers ions sur la vitesse de décomposition de la quinone. Il s'agit ici d'un ion divalent et, à ce propos, nous devons attirer l'attention sur ce fait, observé par Biilmann, que l'électrode à quinhydrone avec une solution de citrates comme liquide électrolyte est très sujette à s'altérer; il se peut qu'ici encore on ait affaire à une décomposition analogue de la quinone.

b. Titrage de l'Hydroquinone.

La teneur en hydroquinone fut titrée en solution alcaline de bicarbonate, saturée d'acide carbonique, par addition d'un excès d'iode dissous dans une solution d'iodure de potassium, puis titrage en retour à l'hyposulfite en présence de l'amidon. Pour étalonner la solution d'hyposulfite, on s'est servi de quantités d'hydroquinone exactement pesées.

Dans le but de vérifier le degré de précision de cette méthode en présence de quinone, de sel etc., nous avons pratiqué un certain nombre d'essais, où une même solution d'hyposulfite fut étalonnée, dans des conditions différentes, sur des proportions d'hydroquinone pesées. Les résultats sont inscrits au Tableau 9, qui ne demande pas d'explication ultérieure.

Il appert de ce Tableau que le titre de la solution d'hyposulfite est toujours le même, que la liqueur titrée soit exempte de sel ou qu'elle en contienne; il est également indifférent que l'étalon même soit la quinhydrone ou une quelconque des préparations d'hydroquinone à notre disposition. De plus, il ressort du tableau que l'addition de quinone donne un titre inférieur à celui obtenu sans quinone. La différence se trouve d'autant plus considérable que la dose de quinone a été plus forte; ce qui probablement trouve son explication dans l'observation, mentionnée plus haut, que les solutions de quinone, même assez récentes, renferment de l'hydroquinone ou un corps titrable comme cette

[1]) La décomposition se fait un peu plus lentement dans ce dernier.

Tableau 9. Essais de Contrôle relatifs au Titrage de l'Hydroquinone.

Hydroquinone employée	Quantité d'hydroquinone employée mg.	Teneur en quinone de la solution mg.	D'autres substances dans la solution	Volume de la solution avant titrage cm^3	Durée de repos de la solution avant titrage en heures	Quantité *d'hydroquinone*, en *mg*, à la quelle correspondait 1 cm^3 de solut. d'hyposulfite
Hydroquinone du commerce (deux fois recr. dans l'ean)	63	0	25 cm^3 HCl 0.01 n	25	$1^1/_2$	5.531
	63	0	—	25	25	5.507
—	63	0	25 cm^3 HCl 0.1 n	25	$1^1/_2$	5.521
—	63	0	—	25	25	5.529
la plus pure de Kahlbaum	63	0	—	25	$1^1/_2$	5.509
—	63	0	—	25	25	5.527
commerciale (recristallisée 2 fois)	63	0	50 cm^3 HCl 0.01 n	50	2	5.522
Quinhydrone	63	62	—	50	2	5.519
la plus pure de Kahlbaum	63	0	—	50	2	5.515
—	63	0	10 cm^3 HCl 0.01 n + 40 cm^3 NaCl 3 n	50	2	5.515
—	63	50	50 cm^3 HCl 0.01 n	50	2	[5.530]
—	63	50	20 cm^3 HCl 0.01 n + 30 cm^3 NaCl 3 n	50	2	[5.525]
commerciale (deux fois recristallisée dans l'eau)	31	0	50 cm^3 HCl 0.01 n	50	3—4	(5.496)
	31	0	—	50	25	(5.496)
—	31	240	—	50	3—4	[5.367]
—	63	0	—	50	3—4	5.516
—	63	0	—	50	25	5.525
—	63	160	—	50	3—4	[5.478]
—	94	0	—	50	3—4	(5.545)
—	94	0	—	50	25	(5.551)
—	94	0	—	50	50	(5.542)
—	94	80	—	50	3—4	[5.528]
					Moyenne...	5.520

dernière. Les titres de l'hyposulfite relatifs aux essais en question sont, dans la dernière colonne de ce tableau, mis entre crochets.

Il est un autre point encore où le titrage de l'hydroquinone présente des difficultés, en ce sens que le titre de l'hyposulfite

se trouve d'autant plus bas que la quantité d'hydroquinone employée est plus faible. Il ressort du tableau que, si elles ne sont pas très grandes, les différences sont au moins appréciables, et, dans nos différents essais, nous avons donc, en étalonnant le liquide de titrage, eu soin d'user de sensiblement la même quantité d'hydroquinone que celles présentes dans les analyses dont il s'agit. Les solutions d'hydroquinone sont plus stables que celles de quinone; aussi le tableau montre-t-il que le titre trouvé de l'hyposulfite ne change pas quand la solution d'hydroquinone utilisée pour l'étalonnage est âgée d'un ou deux jours.

La moyenne a été calculée de tous les étalonnages avec la quantité d'hydroquinone habituelle, env. 63 mg, sans quinone; l'écart d'avec cette moyenne est bien au-dessous de $^1/_2$ pour cent, et même les résultats correspondant aux essais faits avec 31 ou 94 mg d'hydroquinone (ceux marqués par des parenthèses dans le tableau) ne s'écartent de la moyenne que de $^1/_2$ pour cent, au plus.

c. Pureté des Préparations de Quinhydrone.

En ce qui concerne nos préparations de quinone et d'hydroquinone, nous ne les avons soumis à aucune autre épreuve relative au degré de pureté que celle constituée par la concordance des résultats de l'étalonnage. Il n'en est pas tout à fait de même pour les préparations de quinhydrone. Ainsi qu'il a été dit déjà dans le deuxième chapitre (v. p. 15), nous avons rencontré, dans la détermination de u et du produit $k_2 \cdot F$, des difficultés que nous pensons devoir attribuer, en grande partie, aux préparations de quinhydrone employées.

Effectivement, un examen approfondi des préparations de quinhydrone à notre disposition nous a permis de constater que, quoique après dissolution dans l'acide chlorhydrique normal au 1/100 elles aient toutes donné des électrodes ayant la même tension[1]), ces préparations possédaient des solubilités différentes. Les résultats d'une telle série d'essais de solubilité sont réunis dans le Tableau 10.

Des diverses préparations qui ont servi aux expériences relatées ci-dessus, les deux premières avaient été faites dans le Laboratoire de Chimie de l'Université, les autres dans le Laboratoire Carlsberg.

[1]) Les tensions se trouvent rassemblées dans le Tableau 1.

Tableau 10. Solubilité de diverses Préparations de Quinhydrone dans l'acide chlorhydrique, à 18° ± 0.1°.

Marque de la Quinhydrone	Durée de rotation, en heures	Composition de la solution saturée (Mol. par litre de solution)	
		Quinone A	Hydroquinone B
U. k. L. I	4	0.01426	0.01418
U. k. L. II	4	0.01367	0.01362
	19	0.01371	0.01392
C. L. I	4	0.01276	0.01267
	4	0.01264	0.01263
C. L. I a	4	0.01297	0.01293
C. L. II	4	0.01301	0.01302
C. L. III	4	0.01291	0.01292
	19	0.01302	0.01309
C. L. IV	4	0.01295	0.01300
	19	0.01298	0.01307

U. k. L. I et II ont été préparées d'après A. Valeur[1]), en mélangeant ensemble des solutions alcooliques (95 % d'alcool) des deux composants de la quinhydrone, et en employant le double de la proportion calculée d'hydroquinone. Pour préparer C. L. I et I a, nous avons procédé de la même manière, mais en employant des rapports équimolaires entre les deux composants de la quinhydrone. Enfin, C. L. II et IV ont été obtenues par dilution de l'eau-mère provenant, respectivement, de C. L. I et de I a, avec des volumes égaux d'eau, puis addition d'une solution alcoolique de la moitié de la quantité d'hydroquinone utilisée en premier lieu. Tous les précipités se sont déposés sous forme nettement cristalline; après deux ou trois heures de séjour en eau glacée, ils ont été essorés à la trompe et lavés soigneusement à l'alcool de la même concentration que celui dans lequel la précipitation avait eu lieu. Finalement, on a desséché les préparations à l'air le plus rapidement possible.

D'après les deux dernières colonnes verticales du tableau, il faut admettre que les préparations contiennent, en proportions équimolaires, de la quinone et de l'hydroquinone, ou en tout cas des corps titrables de la même manière que celles-ci, ce qui s'accorde parfaitement avec l'observation mentionnée plus haut,

[1]) A. Valeur: Annales de Chimie et de Physique 7, **21**, 547 (1900).

d'après laquelle, employées dans l'électrode à quinhydrone, elles donnent la même tension. On voit en outre, que la solubilité de U. k. L. I et II, et surtout de la première de ces préparations, est notablement plus grande que la solubilité des autres; pourtant, même parmi ces dernières elle se montre sujette à de plus fortes variations qu'on ne devrait s'y attendre d'après la précision avec laquelle les expériences ont été exécutées.

Il n'est guère probable que ces divergences soient attribuables à une saturation incomplète dans les essais où la solubilité a été la moindre, car les essais à rotation de 19 heures ont donné sensiblement les mêmes valeurs que ceux avec 4 heures de rotation, bien que dans tous ceux à longue durée de rotation la quantité d'hydroquinone accuse un léger accroissement, dû sans doute à la décomposition susmentionnée de la quinone avec formation de substances titrables à la façon de l'hydroquinone.

Il se peut qu'il s'agisse ici de diverses modifications cristallines de la quinhydrone, et que l'état d'équilibre définitif ne s'établisse que lentement quand c'est la modification la moins soluble qui entre en jeu. Il y a cependant aussi possibilité qu'il ne s'agisse que de quinhydrone de degrés de pureté différents, et que parmi ces préparations les plus difficilement solubles soient les plus pures. Cette hypothèse se trouve appuyée par les petits essais suivants:

Env. 0ᵍ.2 de chacune des préparations U. k. L. I, C. L. I, II et III, fut trituré dans de petites capsules de porcelaine; ensuite, on les chauffa pendant quelques heures au bain-marie bouillant, en triturant à plusieurs reprises. La température élevée a pour effet de décomposer la quinhydrone; la quinone s'évapore, tandis que l'hydroquinone reste intacte. Les préparations de quinhydrone difficilement solubles soumises à ce traitement ont fourni une hydroquinone d'un blanc légèrement teinté de gris-violet, tandis que la préparation U. k. L. I laissait une hydroquinone jaune brun.

Au reste, la question de la pureté n'a qu'une importance secondaire au point de vue de l'électrode originelle de Biilmann, attendu que toutes les préparations de quinhydrone examinées, après dissolution dans l'acide chlorhydrique normal au 1/100, ont montré la même tension; cependant, il n'est pas certain qu'elles se comportent toutes de même vis-à-vis de solutions à plus forte teneur en électrolytes, et l'on ignore également si

les électrodes sans «erreur de sel» qui, comme on l'a vu dans le troisième chapitre, peuvent être construites par saturation de la liqueur électrolyte avec de la quinhydrone et un des composants de cette dernière, donneront le même potentiel, quelle que soit la nature de la quinhydrone employée.

Nous n'avons pas jusqu'ici eu l'occasion de soumettre ces questions à des recherches plus approfondies; mais, en poursuivant ce travail, l'un de nous, M. Linderstrøm-Lang, ne manquera pas d'y diriger son attention.

Mars 1921.

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JOHS. SCHMIDT:

RACIAL INVESTIGATIONS.

VII. ANNUAL FLUCTUATIONS OF RACIAL CHARACTERS IN *ZOARCES VIVIPARUS* L.

BY

JOHS. SCHMIDT.

I. Introduction.

On statistic investigation of the *Zoarces* populations of northern Europe it was found that a great number of very well characterised "races" could be distinguished, on the basis of differences in the average numerical values of various organs (vertebrae, fin-rays, pigment spots) (Johs. Schmidt 1917. 1.). Later, it was found possible to demonstrate, by experiment and investigation of the offspring that we have here to deal with hereditary qualities, i. e. qualities determined by internal factors. It has also been found, however, that those average values whereby we distinguish race from race (the "racial characters«) can, to a certain, not inconsiderable extent, be altered by "external" factors (Johs. Schmidt, 1917, 2 and 1920).

We know, then, already, that external factors can occasion fluctuations in the average values of the different characters in a population. In the present paper, we shall consider somewhat more closely the fluctuations thus caused by external influences.

We shall first observe a single *Zoarces* population under varying external conditions. When the conditions are changed the average values will be seen to fluctuate, and our task then will be to ascertain how far the different characters agree or differ in their fluctuations.

We shall then proceed to compare several *Zoarces* populations in respect of a single character. Having had several such populations under observation during a period of some years, we have now abundant material whereby to determine the annual fluctuations of the average values concerned.

Finally, mention will be made of some laboratory exper-

iments with the common trout (*Salmo trutta*). These experiments were undertaken with a view to showing the importance of external factors (temperature) in connection with fluctuations in the average values of certain characters in a large number of individuals having both parents in common.

The *Zoarces* populations investigated are from Station 31 (Nakkehage) at the mouth of the Ise Fjord, Station 34 (Langholm) at the base of Roskilde Fjord, and from Kjels Nor, at the southern extremity of Langeland. The greater part of the material has been dealt with by Hr. mag. scient. Vilh. Ege and myself. In addition, Hr. cand. mag. A. Strubberg and Hr. stud. mag. C. A. Jørgensen have taken part in the research. The age determinations of the Kjels Nor material are by Hr. Dr. phil. Ö. Winge, the calculations by Frk. Kirstine Smith. D. Sc. To all those who have assisted me in the investigations, I tender my best thanks.

II. Different Characters in the same Population. Their Fluctuations compared.

When a change in the external conditions of a population causes the average value of this or that character to rise, what happens in regard to the other characters? If, for instance, the number of vertebrae is increased, will the number of rays in the pectorals also increase?

In considering this question, we have recourse to material from two distinct series of investigations. Firstly, there are the transplantation experiments which we carried out in 1916 and 1917, by placing population samples under conditions differing from those under which the population in question normally lives. By comparing the offspring of transplanted individuals with the offspring developed simultaneously under external conditions natural to that population, it can be seen whether the values of the different characters fluctuate in the same direction. And secondly, we have our analyses of the same population extending over a series of years, enabling us to follow and compare the fluctuations of the various characters from one year to another.

A. Transplantation Experiments.

I have in a previous paper (1920) already referred to these transplantation experiments, which were undertaken in 1916 and

1917. They consisted in the transference of a great number of specimens from Station 31 and 34 to large chests submerged in an enclosed piece of water in the vicinity of Station 31, but where the natural conditions are essentially different from those at this station. The transplanted specimens bred and developed offspring while in captivity, and the young were then examined together with offspring developed under normal conditions.

The following characters were investigated: No. of vertebrae ("Vert."), no. of rays in right pectoral fin (Pd), in left pectoral fin (Ps), and of pigment spots on the forepart of the dorsal fin (Pigm. D 1). The results are shown in Table I[1]) and again graphically in Fig. 1.

Table I a. Transplantation Experiments. Stat. 31 1916.

	Non-transplanted	Transplanted	Transplanted ÷ Non-transplanted
Vert. . .	a (123 Dec.) = 113.139 ± 0.089	a (27 Dec.) = 114.819 ± 0.212	+ 1.680
Pd . . .	a (122 Dec.) = 19.025 ± 0.024	a (27 Dec.) = 18.444 ± 0.085	÷ 0.581
Ps . . .	a (121 Dec.) = 19.066 ± 0.023	a (27 Dec.) = 18.470 ± 0.090	÷ 0.596
Pigment	a (87 Dec.) = 13.659 ± 0.075	a (27 Dec.) = 13.189 ± 0.123	÷ 0.470

Table I b. Transplantation Experiments. Stat. 31 1917.

	Non-transplanted	Transplanted	Transplanted ÷ Non-transplanted
Vert. . .	a (177 Dec.) = 113.248 ± 0.075	a (92 Dec.) = 114.452 ± 0.112	+ 1.204
Pd . . .	a (176 Dec.) = 19.102 ± 0.019	a (92 Dec.) = 18.739 ± 0.049	÷ 0.363
Ps . . .	a (175 Dec.) = 19.154 ± 0.020	a (92 Dec.) = 18.786 ± 0.048	÷ 0.368
Pigment	a (127 Dec.) = 13.502 ± 0.067	a (78 Dec.) = 13.182 ± 0.081	÷ 0.320

[1]) After the average values, their probable error is here shown, not, as in previous papers, their probable fluctuation, which is arrived at by multiplying the probable error by 5.

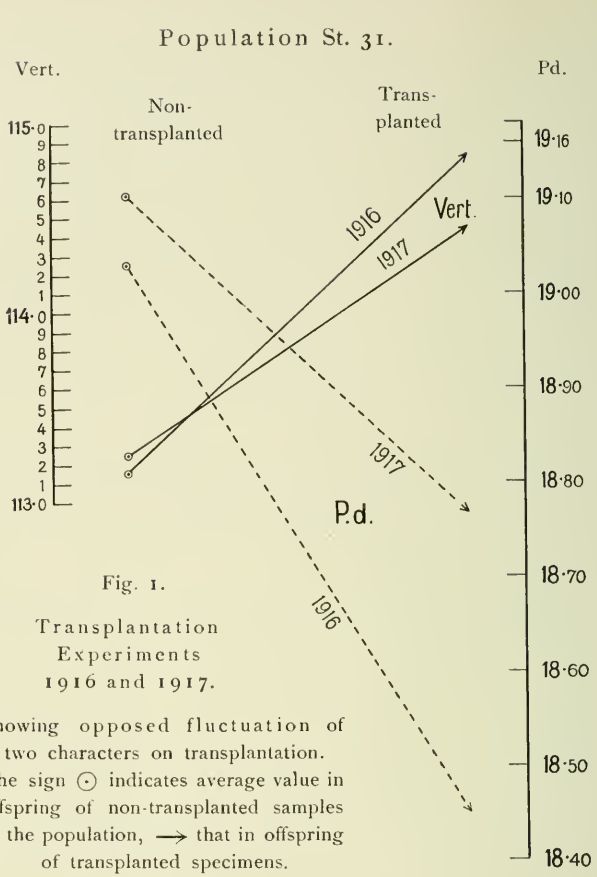

Fig. 1.

Transplantation Experiments 1916 and 1917.

Showing opposed fluctuation of two characters on transplantation. The sign ⊙ indicates average value in offspring of non-transplanted samples of the population, ⟶ that in offspring of transplanted specimens.

It will be noticed that the averages for the different characters have altered considerably as a result of the altered environment on transplantation. The number of vertebrae has increased, whereas the number of pectoral rays and that of the pigment spots have decreased. The symptoms are distinct in all three cases, and tend in the same direction in both years.

We find, then, that an increase in the number of vertebrae does not involve an increase in the values for the other characters; in a word, the characters do not fluctuate alike.

An examination of 7 successive year-classes of this population (from Stat. 31) has enabled us to discern fluctuations of no inconsiderable extent in the averages for the different characters, greatest in regard to the pectoral rays, least in the case of the vertebrae. In no one of these 7 years did the average number

of vertebrae exceed 113.3, nor did the average number of rays in the pectoral fins fall below 19.0[1]). It appears, then, that the fluctuations occasioned by transplantation far exceed those found in the population under normal conditions. I am not in a position to define precisely the nature or extent of the alteration in environment due to transplantation here. It is beyond doubt, however, that the transplanted individuals were living in water of higher temperature and lower salinity than is normal for the population (cf. Johs. Schmidt 1920).

B. Analysis of 10 successive year-classes of the same population.

The population here investigated is from Station 34, at the base of Roskilde Fjord, the year-classes being those of 1912—1921. By "year-class 1921" I understand the class born during the winter of 1920—21. In addition to the characters noted above, in the transplantation experiment, a further one, the number of hard rays in the dorsal fin (D. 2) was here included.

Up to now, we have been able to content ourselves with investigation of embryos, which are easier to work with than older fish. In using embryos for such research, one has, moreover, the advantage of being certain beforehand that all the specimens employed belong to the same year-class D. 2, however, — no. of hard rays in the dorsal — cannot be determined with certainty in embryos, or at any rate, not until they are so far grown as to be very near birth. In order, therefore, to include this character, we were obliged to have recourse to older specimens, which, however, involved age-determination of every single individual employed. Fortunately, age-determinations of *Zoarces* — arrived at, as usual, by counting the rings in scales or otoliths — are comparatively easy. And furthermore, we have always had at our disposal an abundance of material which enabled us to discard specimens whose age appeared in any degree uncertain.

The result of this very extensive piece of research, which was carried out by Hr. mag. scient. Vilh. Ege, is shown, in highly condensed form, in Table II and Fig. 2. Closer examination of the data here given will be found to several points of interest.

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[1]) The values found for the different years are noted in Kirstine Smith 1921.

Table II a. Stat. 34. Vert.

Year-class	1912			1913			1914			1915			1916		
	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ
Samples															
Oct. 1915.	43	107.837±.181	1.758	72	107.847±.157	1.976	99	107.949±.133	1.955	»	»	»	»	»	»
June 1916.	»	»	»	»	»	»	»	»	»	433	108.224±.066	2.039	64	108.094±.183	2.173
Dec. 1916.	»	»	»	»	»	»	41	107.976±.222	2.103	346	108.058±.078	2.153	25	108.520±.410	3.043
May 1917.	»	»	»	»	»	»	15	108.333±.284	1.633	131	108.008±.122	2.074	297	108.077±.077	1.965
Nov. 1917.	»	»	»	»	»	»	»	»	»	138	108.232±.107	1.865	201	108.065±.091	1.908
Oct. 1918.	»	»	»	»	»	»	11	108.455±.368	1.809	93	108.086±.139	1.987	326	108.396±.072	1.937
Nov. 1919.	»	»	»	»	»	»	»	»	»	»	»	»	81	107.790±.149	1.986
Nov. 1920.	»	»	»	»	»	»	»	»	»	»	»	»	17	107.765±.228	1.393
Total..	43	107.837±.181	1.758	72	107.847±.157	1.976	166	108.024±.102	1.948	1141	108.138±.041	2.053	1011	108.161±.042	1.989
Embryos..	»	»	»	»	»	»	»	»	»	»	»	»	1940	108.270±.067	»

Year-class	1917			1918			1919			1920			1921		
	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ
Samples															
Oct. 1915.															
June 1916.															
Dec. 1916.															
May 1917.															
Nov. 1917.															
Oct. 1918.	195	107.687±.099	2.051	210	107.714±.093	1.989									
Nov. 1919.	165	107.618±.108	2.050	234	107.603±.086	1.950	356	108.197±.066	1.849						
Nov. 1920.	43	107.791±.215	2.088	141	107.702±.110	1.941	377	108.130±.063	1.821	371	107.779±.066	1.873			
Total..	403	107.670±.069	2.050	585	107.667±.055	1.959	733	108.162±.046	1.834	371	107.779±.066	1.873			
Embryos..	580	107.783±.093	»	730	107.723±.084	»	740	108.312±.073	»	615	108.101±.096	»	750	107.903±0.081	»

Year-class	1912			1913			1914			1915			1916		
	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ
Samples															
Oct. 1915.	42	19.238±.060	.576	75	19.067±.050	.644	100	19.170±.043	.637	»	»	»	»	»	»
June 1916.	»	»	»	»	»	»	»	»	»	438	18.975±.019	.575	65	19.462±.055	.663
Dec. 1916.	»	»	»	»	»	»	44	19.000±.066	.647	348	19.017±.022	.598	25	19.480±.079	.586
May 1917.	»	»	»	»	»	»	17	19.000±.100	.612	142	18.930±.032	.567	304	19.411±.022	.573
Nov. 1917.	»	»	»	»	»	»	»	»	»	140	19.121±.036	.640	203	19.434±.027	.580
Oct. 1918.	»	»	»	»	»	»	13	19.077±.120	.641	101	18.941±.040	.597	335	19.433±.022	.605
Nov. 1919.	»	»	»	»	»	»	»	»	»	»	»	»	82	19.537±.047	.632
Nov. 1920.	»	»	»	»	»	»	»	»	»	»	»	»	17	19.412±.117	.712
Total . .	42	19.238±.060	.576	75	19.067±.050	.644	174	19.103±.033	.637	1169	18.997±.012	.592	1031	19.437±.013	.597
Embryos .	»		»	»		»	»	»	»	»	»	»	1970	19.387±.020	»

Year-class	1917			1918			1919			1920			1921		
	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ
Samples															
Oct. 1915.															
June 1916.															
Dec. 1916.															
May 1917.															
Nov. 1917.															
Oct. 1918.	193	19.249±.030	.613	228	18.934±.027	.594									
Nov. 1919.	166	19.181±.031	.586	236	18.945±.026	.599	357	19.160±.022	.622						
Nov. 1920.	43	19.186±.068	.664	141	19.028±.035	.620	379	19.177±.019	.561	370	19.530±.021	.598			
Total . .	402	19.214±.020	.607	605	18.960±.017	.602	736	19.168±.015	.591	370	19.530±.021	.598			
Embryos .	590	18.910±.032	»	735	18.973±.023	»	740	19.154±.019	»	615	19.598±.025	»	750	19.239±.022	»

Table II c. Stat. 34. Ps.

Year-class	1912			1913			1914			1915			1916		
	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ
Samples															
Oct. 1915.															
June 1916.	»	»	»	»	»	»	»	»	»	452	19.013±.020	.628	65	19.554±.055	.662
Dec. 1916.	»	»	»	»	»	»	44	18.977±.051	.505	348	19.078±.023	.624	25	19.520±.079	.586
May 1917.	»	»	»	»	»	»	»	»	»	141	18.979±.036	.626	300	19.453±.023	.591
Nov. 1917.	»	»	»	»	»	»	»	»	»	140	19.143±.035	.607	202	19.455±.028	.582
Oct. 1918.	»	»	»	»	»	»	14	19.000±.100	.555	101	19.010±.040	.592	332	19.467±.022	.588
Nov. 1919.	»	»	»	»	»	»	»	»	»	»	»	»	82	19.549±.044	.591
Nov. 1920.	»	»	»	»	»	»	»	»	»	»	»	»	16	19.500±.123	.730
Total..	»	»	»	»	»	»	58	18.983±.045	.513	1182	19.043±.122	.622	1022	19.475±.013	.594
Embryos..	»	»	»	»	»	»	»	»	»	»		»	1970	19.432±.020	»

Year-class	1917			1918			1919			1920			1921		
	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ
Samples															
Oct. 1915.															
June 1916.															
Dec. 1916.															
May 1917.															
Nov. 1917.															
Oct. 1918.	202	19.277±.029	.617	229	18.956±.028	.627									
Nov. 1919.	163	19.282±.031	.583	236	19.025±.027	.618	356	19.197±.021	.596						
Nov. 1920.	43	19.163±.071	.688	141	19.043±.035	.608	378	19.259±.021	.611	371	19.544±.021	.588			
Total..	408	19.267±.020	.611	606	19.003±.017	.619	734	19.229±.015	.604	371	19.544±.021	.588			
Embryos..	580	18.960±.030	»	735	19.018±.023	»	740	19.220±.019	»	615	19.626±.025	»	750	19.288±.023	»

Table II d. Stat. 34. D_2.

Year-class	1912			1913			1914			1915			1916		
	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ
Samples															
Oct. 1915	42	5.905 ± .134	1.284	72	6.458 ± .102	1.288	95	4.937 ± .092	1.335	»	»	»	»	»	»
June 1916	»	»	»	»	»	»	»	»	»	405	6.714 ± .041	1.234	56	5.821 ± .084	.936
Dec. 1916	»	»	»	»	»	»	44	5.159 ± .116	1.140	327	6.798 ± .042	1.139	23	6.043 ± .108	.767
May 1917	»	»	»	»	»	»	14	4.786 ± .161	.893	133	6.714 ± .073	1.247	277	5.830 ± .046	1.137
Nov. 1917	»	»	»	»	»	»	»	»	»	134	6.627 ± .071	12.12	192	5.854 ± 0.55	1.121
Oct. 1918	»	»	»	»	»	»	14	5.286 ± .304	1.684	98	6.837 ± .092	1.352	325	5.880 ± .044	1.168
Nov. 1919	»	»	»	»	»	»	»	»	»	»	»	»	78	5.859 ± .100	1.307
Nov. 1920	»	»	»	»	»	»	»	»	»	»	»	»	16	5.625 ± .149	.885
Total . .	42	5.905 ± .134	1.284	72	6.458 ± .102	1.288	167	5.012 ± .067	1.285	1097	6.739 ± .025	1.216	967	5.855 ± .025	1.136

Year-class	1917			1918			1919			1920		
	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ
Samples												
Oct. 1915 . . .												
June 1916 . . .												
Dec. 1916 . . .												
May 1917 . . .												
Nov. 1917 . . .												
Oct. 1918 . . .	195	5.841 ± .060	1.235	220	6.614 ± .054	1.198						
Nov. 1919 . . .	159	5.881 ± .070	1.309	231	6.615 ± .054	1.210	326	6.540 ± .045	1.199			
Nov. 1920 . . .	43	5.721 ± .128	1.241	129	6.574 ± .073	1.223	366	6.410 ± .039	1.118	359	5.337 ± .041	1.156
Total . .	397	5.844 ± .043	1.264	580	6.605 ± .034	1.206	692	6.471 ± .030	1.158	359	5.337 ± .041	1.156

Table II e. Stat. 34. Pigm. D_1.

Year-class	1912			1913			1914			1915			1916		
	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ
Samples															
June 1916.	»	»	»	»	»	»	»	»	»	282	12.691±.061	1.516	33	12.303±.189	1.610
Dec. 1916.	»	»	»	»	»	»	»	»	»	265	12.728±.071	1.715	»	»	»
May 1917.	»	»	»	»	»	»	»	»	»	99	12.636±.130	1.914	218	12.454±.079	1.723
Nov. 1917.	»	»	»	»	»	»	»	»	»	95	12.863± 123	1.772	126	12.357±.096	1.602
Oct. 1918.	»	»	»	»	»	»	»	»	»	82	12.817±.130	1.751	255	12.475±.076	1.805
Nov. 1919.	»	»	»	»	»	»	»	»	»	»	»	»	»	12.507±.132	1.655
Total..	»	»	»	»	»	»	»	»	»	823	12.729±.040	1.683	703	12.442±.044	1.717
Embryos..	»	»	»	»	»	»	»	»	»	»		»	»	»	»

Year-class	1917			1918			1919			1920		
	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ	n	a ± P. E. A.	σ
Samples												
June 1916 . . .												
Dec. 1916 . . .												
May 1917 . . .												
Nov. 1917 . . .												
Oct. 1918 . . .	157	12.446 ± .099	1.831	183	12.525 ± .088	1.760						
Nov. 1919 . . .	124	12.468 ± .113	1.863	193	12.306 ± .079	1.625	290	12.500 ± .068	1.705			
Total . . .	281	12.456 ± .074	1.842	376	12.412 ± .059	1.693	290	12.500 ± .068	1.705	»	»	»
Embryos. . .	1180	12.404 ± .083	»	1430	12.397 ± .067	»	1170	12.448 ± .060	»	1220	11.911 ± .069	»

The graph in Fig. 2 embraces vertebrae, rays in right pectoral and hard rays in dorsal for the years 1912—1921. We saw from the transplantation experiments (Fig. 1) that the no. of vertebrae and no. of pectoral rays did not fluctuate in the same direction, the increase in no. of vertebrae on transplantation being accompanied, both in 1916 and 1917, by a decrease in the no. of pectoral rays. As far as the experiment went, it might seem as if the two characters tended in opposite directions, the one rising as the other fell. A glance at Fig. 2, however, shows us that no such rule can be established, since we here find the two features for the 1920 year-class, as in the transplantation experiments, fluctuating in opposite directions, and the no. of vertebrae and no. of pectoral rays moving in the same direction for the year classes of 1916 and 1917.

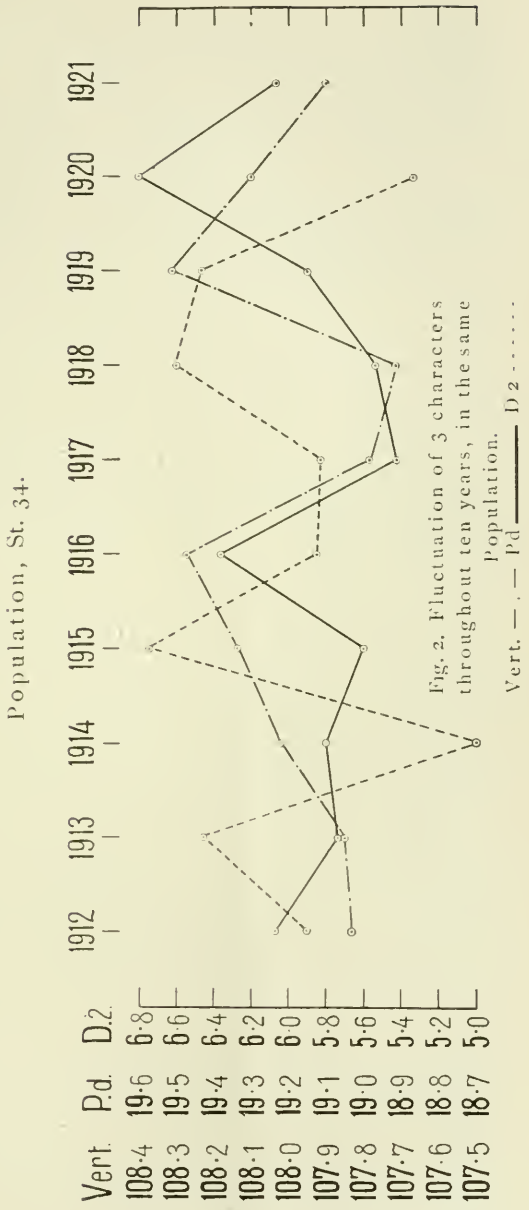

Fig. 2. Fluctuation of 3 characters throughout ten years, in the same Population.
Vert. — . — Pd ——— D 2 .....

A further scruting of the curves for the ten years will show that something similar applies to the hard rays in relation to

the other characters, and we can, as the final result of our investigations, lay down the fact that the different characters are not bound to fluctuate alike from one year to another. We may find one year-class with high values for no. of vertebrae and hard rays, while the next is altogether different in this respect, having, say, a high value for the hard rays and a low number of vertebrae, etc. etc. There is no fixed rule for such cases.

By following the curves for the different characters in Fig. 2,

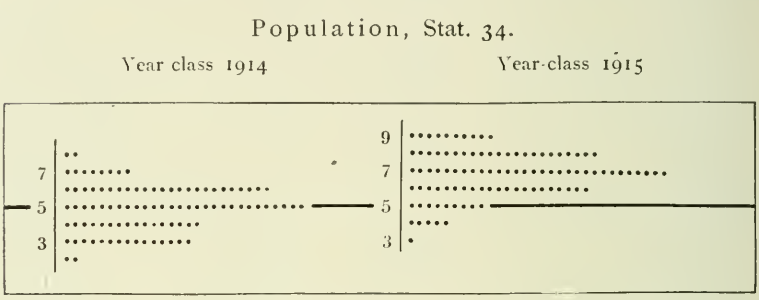

Fig. 3. No. of hard rays (D. 2) in two year-classes of the same population.

it will be seen that very considerable differences may exist between the separate year-classes. When we find, for instance, that the average for hard rays can vary from 5.012 to 6.739, this must be regarded as a fluctuation of extraordinary extent, and the same applies to the pectoral rays, where the lowest and highest values found in these ten years are 18.960 and 19.626. The great difference is, perhaps, especially apparent when the year-classes in question are shown graphically one beside the other, as in Fig. 3. The differences here are so great, that one would be more inclined to fancy it was a case of two different "races", instead of merely two year classes of the same population. It is worth noting, by the way, that the curves do not by any means exhibit an even rise from the lowest values to the highest, or vice versa. We find, for instance, that the hard rays are at their lowest in 1914, rising then with a violent ascent to their maximum in 1915.

From Tables II a-e it will be seen that during the past years, samples of the population were taken once or twice a year for purposes of analysis. These samples were in each case resolved into their component year-classes, each of which was

analysed separately. To quote an example, the 1916 year-class was thus analysed at no fewer than 6 different stages of its life, first in the autumn of 1915, when it was still in the embryo stage, later as a 1- 2- 3- and 4-year-old fish, and finally in the autumn of 1920, when it was 5 years old.

What agreement do we now find between these analyses of the 1916 year-class at different stages in its life as a class? The question is obviously one of great importance to the scope of our investigations. Closer examination of the tables shows that the figures are extremely satisfactory in this respect; the analyses of a year-class gave, in fact, the same result, whether made in its first year or subsequently. Some interesting conclusions may be drawn from this. We learn that our age-determinations are correct, a point of importance extending beyond the subject in hand, since we are thus enabled, by means not hitherto employed to furnish an always welcome proof that the age-determinations of fish, which are used nowadays to so large an extent, rest on a solid foundation.

It is also of importance to note that we find, year after year, at the same place, the same year-class characterised by the natural conditions prevailing at the time of its "sensitive period" in the embryo stage, and mingled, also, with other year-classes, each of which is similarly marked by the environment in which its own sensitive period was passed.

This even mixture of year-classes giving, year after year, on repeated analysis, the same average values, we may regard as an indication of the fact that our population is highly stationary; no year-class appears to desert the particular locality where it came into the world, nor does any such class, as far as can be seen, suffer alteration in its composition by selection through the dying-off or emigration of certain biotypes.

The total impression then, resulting from our investigation is, to my mind, that we have here a population in which the mixture of biotypes has come to a standstill, and remains constant, whereas the phænotype is continually altering from year to year, as a result of the change in natural conditions from one year to another.

III. The same character in two populations. Comparison of its fluctuations over a series of years.

A. Number of rays in the pectoral fins (Pd and Ps).

We will first consider the characters Pd. and Ps., i. e. the number of rays in right and left pectoral fin, keeping for the present to the much-used Stations 31 and 34. The former, it will be remembered, is situate close to the mouth of the Ise Fjord, the latter at the base of Roskilde Fjord. The year-classes investigated are those of 1915—1921, and, with exception of the 1915 class from Station 34, the material, which ich very extensive, consists exclusively of embryos.

The number of pectoral rays can be determined with perfect certainty in embryos stained with alizarine (cf. Johs. Schmidt, 1917, 2). The number varies between 17 and 21. Of these five variates, however, 17 is very rare. The figure 21 is somewhat more common, but 19, 20 and 18 are by far the most frequently occurring values. Both right and left pectorals were examined. In contrast to many other species which I have investigated. *Zoarces* has a slightly higher average of rays in the left pectoral fin than in the right.

It should be borne in mind that the two populations here in question are separated one from the other by a distance of some 50 kilometres (cf. Fig. 4). It is not easy to distinguish between them by the number of pectoral rays; they are, however, very dissimilar; there is a constant difference, for instance, of abt. 5 in the average no. of vertebrae. In this respect, I would refer to the papers previously quoted, (Johs. Schmidt, 1917, 1, and 1920). In the latter, I mentioned our transplantation experiments in 1916 with samples from both populations, the result of which was to show that the great difference in the no. of vertebrae was maintained in the offspring, despite the fact that their development took place under the same external conditions.

The result of the 7-year investigation is shown, for each of the populations, in the graph Fig. 5, where the values for right and left pectoral (Pd. and Ps.) are shown in separate figures.

From these, it is distinctly evident that the average value for the same population fluctuates greatly from year to year, and, moreover, that the two pectorals keep together, the left having throughout a slightly higher average than the right.

Of more than ordinary interest, however, is the fact that the annual fluctuations of the average values in the

Fig. 4. Average no. of vertebrae in 11 *Zoarces* populations from the Ise Fjord—Roskilde Fjord area.
The figures by the black spots are station numbers; those in the squares indicate the average no. of vertebrae for the population in question.

two populations are alike for both: when one population shows a high figure, the other does the same, and in like manner with regard to low values.

The fact thus demonstrated obviously warrants two conclusions, viz. 1) that the number of pectoral rays is subject to the

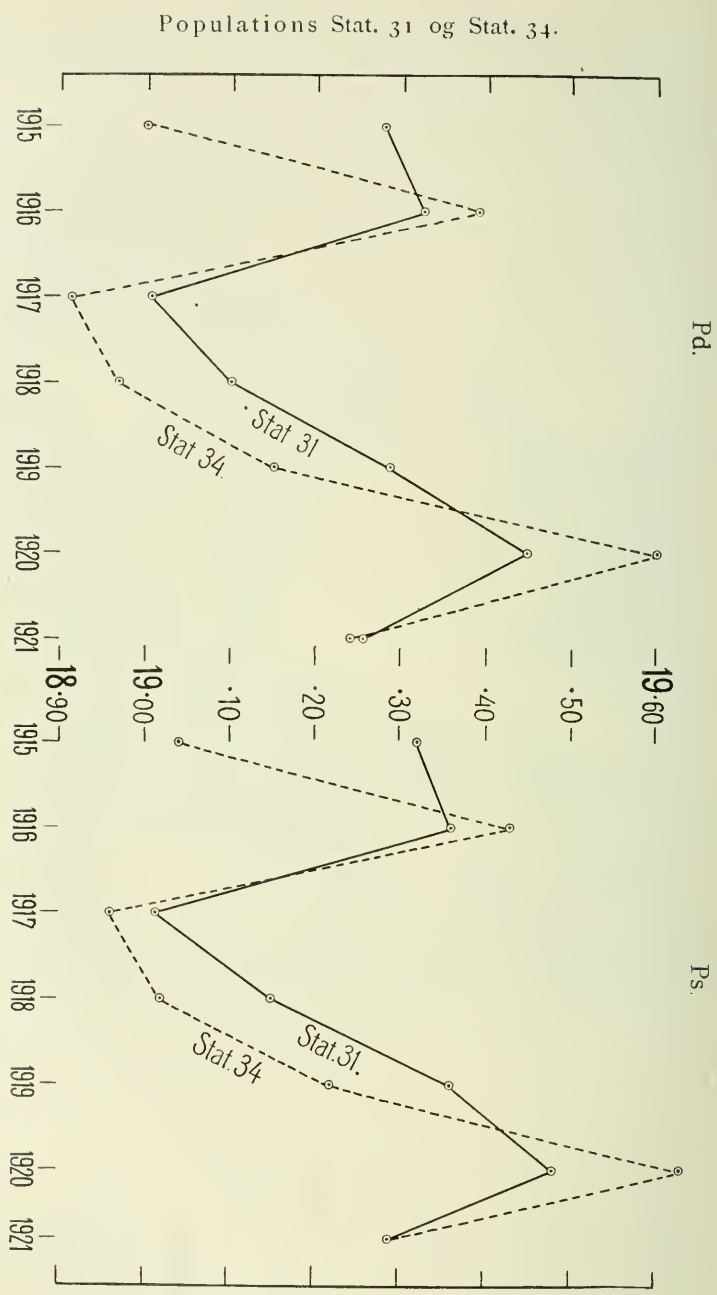

Fig. 5. Showing fluctuations of a character throughout 7 years in two different populations (Stat. 31 og Stat. 34).
The character investigated is the number of rays in right (Pd) and left (Ps) pectoral fin.

influence of external conditions prevailing during the period which precedes formation of the rays themselves, and, 2) that both populations have in this respect been influenced by the same external factor or factors.

Furthermore, it seems as if the factor in question had made itself more markedly felt among the population from the base of Roskilde Fjord, (Stat. 24) since the fluctuations in the values are here more pronounced than in the population from the Ise Fjord (Stat. 31).

B. Number of hard rays in dorsal fin (D. 2).

Of the material from Stations 31 and 34, we have age-determinations only of that from the latter: consequently, the two stations cannot be compared in respect of annual fluctuations in the number of hard rays. We have, however, age-determined material from a third station, viz. Kjels Nor, situate at the southern extremity of Langeland[1]). We can, then, compare the Kjels Nor Station with Station 34 in Roskilde Fjord.

One of the most peculiar features in *Zoarces* is the presence of a small number of hard rays, interpolated in the otherwise soft-rayed dorsal fin. The number of these hard rays has proved to be of great importance in distinguishing between different "races". The *Zoarces* populations of the East Baltic area, for instance, are distinguished by their large number of hard rays, whereas the populations of the North Sea have only a small number of these (Johs. Schmidt 1917, 1, Pl. I).

From Kjels Nor Station, we have investigated the year-classes 1913—1919 inclusive, with the exception of year-class 1916. The result of the investigation for both stations is shown graphically in Fig. 6.

It will be seen from the figure, that the annual fluctuations of the average values go together in the two populations. We may therefore draw the same conclusion as in the case of the pectoral rays, viz., that the two populations must have been under the influence of the same external factor during the sensitive period preceding the formation of their hard rays.

[1]) The position of this station can be seen from Pl. II in my paper 1917. 1. — It lies close to St. 43, which is shown in the chart Pl. II.

On comparing the two figures 5 and 6, however, we find a remarkable difference between them. The pectoral fin curves for the two otherwise very dissimilar populations lie close together, intersecting, indeed, now and again; the curves for the hard rays, on the other hand, are far apart, despite the fact that they fluc-

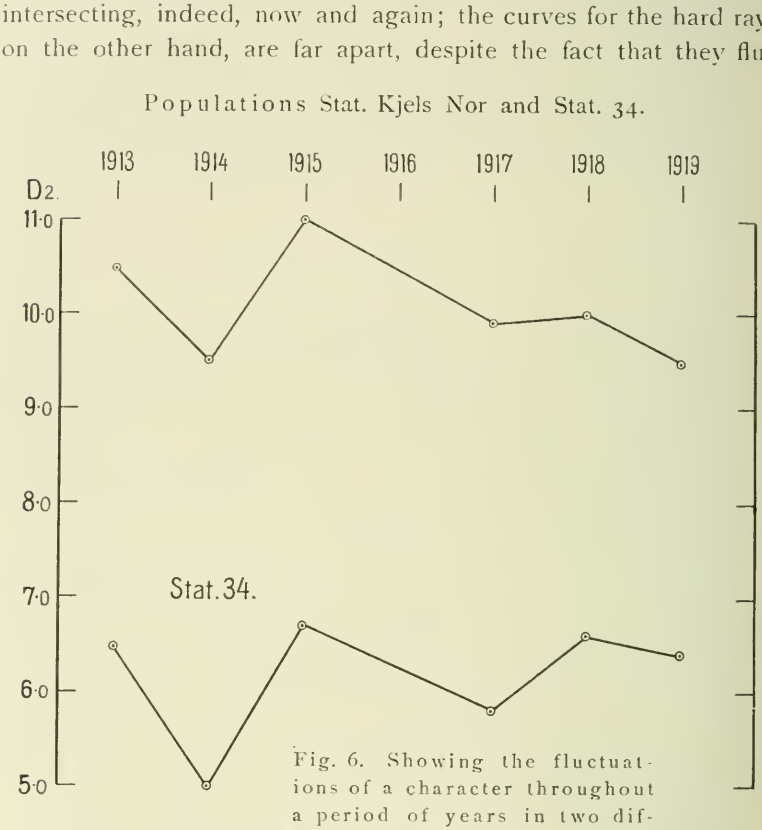

Fig. 6. Showing the fluctuations of a character throughout a period of years in two different populations (Stat. Kjels Nor, upper, and Stat. 34). The character investigated is the number of hard rays in dorsal fin (D. 2). Year-class 1916 was lacking in St. Kjels Nor, and is therefore also omitted for St 34.

tuate together. In the former case, it is phænotypical differences — due to external factors — between the year-classes which predominate; in the latter, though phænotypical differences may no doubt be discerned between the year-classes, they cannot by any means efface the distinct impression of marked genotypical differences — due to internal factors — between the two populations.

IV. Laboratory Experiments with the Common Trout. (*Salmo trutta* L.)

We have, in the foregoing, seen, that the average values for various characters in *Zoarces* can fluctuate greatly from year to year. Further, a comparison of the two populations throughout a series of years has shown that the average for the same character can fluctuate in the same direction in the two populations, from which we concluded that the embryos of both populations must during their sensitive period, have been under the influence of the same external factors.

The mating season for *Zoarces* falls in July, and by the end of September, the embryos have their pectoral rays, for instance, already indicated; consequently, the sensitive period — i. e. the period during which the number is determined — must lie within the time limits mentioned. Nothing more definite, however, is known as to this; we do not know, for instance, how long the period is. The hard rays in the dorsal fin appear later than the other characters investigated, and it is therefore probable, at least, that the sensitive period for this character falls somewhat later than for the others.

Unfortunately, I am unable to explain what are the factors in question. To arrive at the possibility of any such explanation, it would be necessary to have carried out a thorough and detailed investigation of the physical, chemical and biological conditions at the stations concerned during all these years. In view of the fact that the stations (31, 34 and Kjels Nor) lie so far apart as they do, it seems evident that it cannot be merely a case of one or more factors of purely local effect. One is rather inclined to think of a factor such as the temperature, which must be presumed to make itself felt over a greater area, more especially since I have previously been able to show that temperature is of great importance in regard to the number of rays in the pectoral fins of another species (Johs. Schmidt, 1917, 2 and 1919).

I therefore endeavoured to find some connection between the fluctuations of averages for Pd, Ps., and D. 2, as shown in Fig. 5 and 6, and the temperature values during the summer months.

It was there found, that generally speaking, high summer temperatures give a large number of hard rays and a small

number of pectoral rays in the year-class born the following winter. This rule, however, does not always apply, which would seem to suggest the disturbing influence of other factors, whose effect we cannot determine from the data at present available. It is probably a question which can only be answered satisfactorily by laboratory experiments. Up to the present, I have not been able to carry out such experiments with *Zoarces*. I have, however, made an experiment with the common trout (*Salmo trutta*) in the laboratory, which I hope later on to describe at greater length. I mention here a single feature, viz.: the effect of temperature upon the number of vertebrae, in order to show — as bearing upon the *Zoarces* investigations — that we have here to deal with a highly complicated state of things.

The material for this experiment, carried out at Carlsberg Laboratory during the winter of 1917—1918, was obtained by artificial fertilisation of the ova of a single female with the sperma of a single male. All the specimens dealt with had thus both parents in common, which we must suppose would be an advantage when seeking to ascertain the effect of varying external conditions upon a given biotype.

The experiment falls throughout into three series. In the two first, the development of the ova and young took place in running water, the water in the third case being still, but aerated by a constant current of atmospheric air. The difference between Series 1 and 2 was that in the former, the water was introduced directly, whereas in the latter, it had previously passed through the aquaria belonging to Series 1.

The temperatures at which development took place were as follows:

Series 1 . . . 1,9°, 6,5°, 10,4°.
Series 2 . . . 2,3°, 6,7°, 10,2°.
Series 3 . . . 1,9°, 4,0°, 7,0°.

We had thus, in each of the three series, three samples of young, developed at low, intermediate and high temperature. The number of specimens to the sample was round about 50 (varying from abt. 30 to abt. 80), values which, with the slight range of variation here in question, permit of a sufficiently accurate determination of the averages.

Fig. 7 shows directly the result of the experiment. It will

be seen from this, that the average number of vertebrae is high at the lowest temperatures, falling then at the

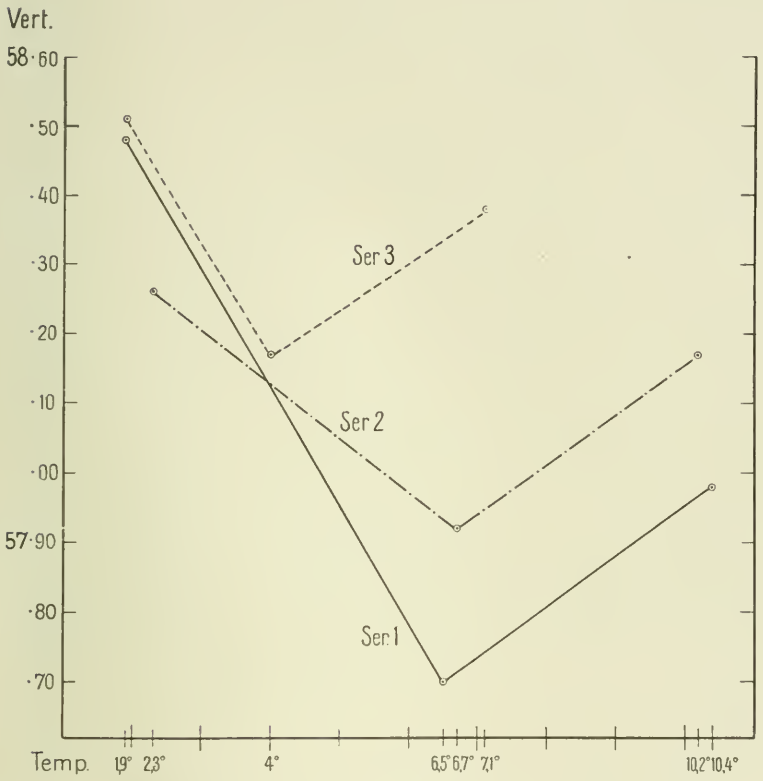

Fig. 7. Laboratory Experiment with the Common Trout (*Salmo trutta* L.). Showing the dependence of average no. of vertebrae upon temperature.

intermediate, to rise once more at the highest temperatures.

Having only tested each series under three temperatures, we are unable to state precisely where lie the maximum and minimum of the curve, but in any case, the result is clear enough, and uniform for all three series.

We find, then, that the question is not altogether simple. Intermediate temperatures gave a small number of vertebrae, but a large number was produced both by the lowest and the highest temperatures operating in the experiment. Should a like state of things apply in the case of *Zoarces*, this in itself would be sufficient to explain the fact that the fluctuations of average

values shown in Figs. 5 and 6 do not always appear to follow the fluctuations of temperature from year to year. Apart from this many other factors may be imagined as possible disturbing influences; further discussion of these, however, lies outside the scope of the present paper.

V. Summary.

There are in northern Europe numerous races of *Zoarces*, which are very well characterised one against another by average differences in the numerical values of certain organs such as vertebrae, fin-rays, etc. Further investigation of the races — or, as I prefor to call them, the populations — has, however, shown that the averages are not constant, but subject to fluctuations which may at times be of some considerable extent. It is with these fluctuations of the average values that the present paper is concerned. The material is derived from three very different populations, two (St. 31 and 34) being from the mouth of the Ise Fjord and the base of Roskilde Fjord respectively, while the third is from Kjels Nor, at the southern extremity of Langeland. The following characters in particular were investigated: no. of rays in pectoral fins (Pd. and Ps.), no. of hard rays in dorsal (D 2) and no. of vertebrae (Vert.). The investigations have now extended over a number of years, covering as many as 10 separate year-classes, and the results distinctly show that the fluctuations in question are due to fluctuations in the environment.

Section II deals first of all with some transplantation experiments carried out in 1916 and 1917. From there it appears that the different characters do not fluctuate alike: simultaneously with an increase in the number of vertebrae, a decrease in the number of pectoral rays took place (Table I and Fig. 1).

Investigation of 10 successive year-classes from the Roskilde Fjord population (St. 34) gave further proof that the different characters do not fluctuate alike (Table II a—e, and Fig. 2).

In Section III, comparison is made between the number of pectoral rays in year-classes 1915—1921 at Stations 31 and 34 (see Fig. 4). It was found that the annual fluctuations in the average values of the two populations keep together. (Fig. 5). The same applies to the number of hard rays in the populations from Roskilde Fjord (St. 34) and Kjels Nor, Langeland, for the

years 1913—1919; during the years with high average for Roskilde Fjord, the Langeland average is also high, and vice versa, low values for both populations in the same years (Fig. 6). From this we may conclude that both populations must have been under the influence of the same external factor during their "sensitive" period, which precedes the formation of the rays in embryo, and furthermore, that the factor or factors in question cannot have been of merely local occurrence. Which factor it is, we cannot say. Generally speaking, however, it would seem that a high temperature during the sensitive period, which in the case of *Zoarces* must fall in the months of July—September, gives the offspring a smaller number of rays in the pectoral fins, and more hard rays in the dorsal. The rule, however, is not without exception, which seems to suggest that the question may be complicated in various ways. In all probability, it can only be solved by laboratory experiments. We have had no opportunity of making such with *Zoarces*, but have done so with the common trout *(Salmo trutta)*. Here, the influence of temperature on no. of vertebrae was tested, by letting a quantity of eggs develop at three different temperatures. The result was, that the lowest number of vertebrae was produced at intermediate temperatures, the value rising both with higher and with lower temperatures (Fig. 7).

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COMPTES-RENDUS

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LES COMPTES-RENDUS
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paraissent par livraisons à des époques indéterminées. A mesure qu'il en paraîtra un nombre suffisant pour faire un volume, les abonnés recevront un titre en même temps qu'une table des matières, avec l'indication de la période qu'embrasse le volume.

JOHS. SCHMIDT:

RACIAL INVESTIGATIONS.

VIII. THE NUMERICAL SIGNIFICATION OF FUSED VERTEBRAE.

BY

JOHS. SCHMIDT.

Investigation of the vertebral column in fishes not unfrequently discloses vertebrae that give the impression of being double i. e. formed through the fusion of two single vertebrae. In endeavouring to aseertain the number of vertebrae — e. g. for statistical or genetic purposes — one is therefore confronted with the question as to how such fused vertebrae should be counted. Are they to be regarded as two vertebrae or as only one? Or are both views erroneous?

In previous works of a statistical nature I have evaded the difficulty by rejecting altogether specimens with fused vertebrae, as I considered that in such works no error of importance could occur when the material under investigation was large and the number of specimens with fused vertebrae small.

When dealing with genetic works, however, investigating the inheritance of the number of vertebrae, the case is more doubtful. By ignoring individuals with fused vertebrae one is running the risk of committing a serious error.

The question has been raised during the investigation regarding the inheritance of vertebrae in the trout, on the basis of which I described the so-called diallel crossing's method (1919, 1 and 2). To avoid overloading the essay I did not on that occasion enter into the question of the significance of the fused vertebrae. I shall now therefore discuss the matter briefly.

In the table are given the results of the first experiment in diallel crossings, wherein 4 female specimens were crossed with 2 male specimens, so that 8 offspring-combinations resulted. These are distinguished by the parents' symbols, the males being

Diallel Crossings with Trout (*Salmo trutta*), Number of vertebrae.

1) Individuals with single, 2) Individuals with double hæmal arch at the 5 last vertebrae.

No. of vertebrae	x a	x b	x c	x d	y a	y b	y c	y d
1) 61	»	»	»	»	»	3	»	»
60	»	»	»	»	46	90	»	6
59	30	95	»	4	280	153	7	160
58	193	98	81	211	90	9	33	193
57	23	»	147	85	»	»	1	9
56	»	»	1	»	»	»	»	»
a_1	58.028	58.492	57.349	57.730	58.894	59.341	58.146	58.443
2) 61-60 (60½)	»	»	»	»	»	2	»	»
60-59 (59½)	»	»	»	»	23	15	»	7
59-58 (58½)	5	19	1	5	28	4	1	37
58-57 (57½)	9	2	27	18	»	»	1	4
a_2	57.857	58.455	57.536	57.717	58.951	59.405	58.000	58.563
+ 2) 1 a_{1+2}	57.109	58.488	57.370	57.729	58.900	59.346	58.140	58.457.

1) Total average... **58.303**. 2) Total average... **58.310**.

marked *x* and *y*, the females *a*, *b*, *c* and *d*. The table thus shows the number of vertebrae in the 8 offspring-combinations.

It is a striking fact (e. g. in comparison with *Zoarces*) that each sample contains very few variates indeed. Thus the combination *xb* has only 2 variates, both occurring with almost equal frequency, namely 58 and 59. In none of the samples do we find more than 4 variates. In *Zoarces* as a rule the individual-offspring samples had 6—8, and even 12, variates as regards the number of vertebrae (*vide* Johs. Schmidt, 1917, p. 317—319).

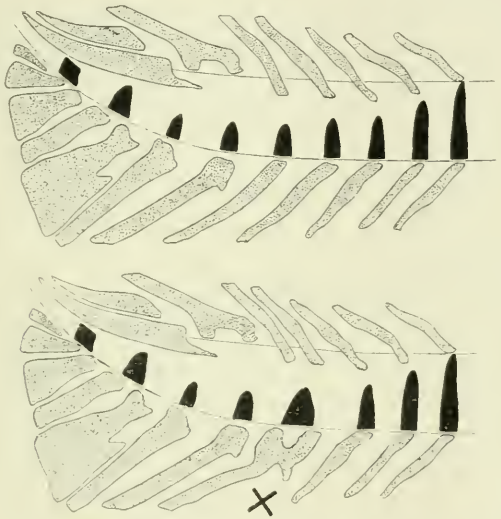

Fig. 1. — End of tail of two trout young (*Salmo trutta*), length ca. 22 mm.; alizarine-staining. — In the lower specimen the fifth vertebra (marked ×) from the end is fused. Notice the longer body, the partly double hæmal and the double neural arch.
Drawing by Vilh. Ege.

The trout then is remarkable for the fact that its individual offspring samples have very narrow limits of variation.

It must however be added that in all 8 samples was found a small number of individuals (average ca. 10 %) whose 5-last vertebra shows a peculiarity impossible to ignore and which places one in doubt as to whether one is dealing with a single or a double vertebra.

In the accompanying figures are depicted the ends of the vertebral column in a specimen of this nature and in a "normal" specimen.

Whilst apparently there is not here, as in other cases of fusion of vertebrae, anything to remark in the body itself, apart from its being longer than usual, the hæmal arch proves to be double in its proximal part, being thus bifurcated (*vide* Fig.); the neural arch is double. The question is now: What value in counting the vertebrae shall be allowed to such a 5-last vertebra with double arch? Is it to be reckoned as 2 or only as 1, or does the truth lie between? This question is obviously of considerable importance when a sample contains many specimens with double hæmal arch.

In order to examine the matter more closely let us imagine two individuals, a male and a female, both homozygous as regards numbers of vertebrae. Let us further imagine that the one has an even, the other an uneven number of vertebrae, e. g. 58 and 59. In such a case one might expect that their offspring would have $58^1/_2$ vertebrae. Reasoning thus one might be allowed to regard it as probable that the 5-last vertebra in question with double hæmal arch would have a value lying between 1 (single vertebra) and 2 (double vertebra).

To test this theory I have experimentally counted it as $1^1/_2$, so that a specimen with 58 normal vertebrae besides the 5-last with double arch, is counted as having $59^1/_2$ vertebrae, etc. etc. A test regarding the accuracy of this assumption is possible in the following manner. If within each of the 8 offspring samples we ascertain the average number of vertebrae in specimens having double arches at the 5-last vertebra, counting this vertebra as $1^1/_2$, the average must coincide with the average for the normal individuals, provided our theory be correct.

Proceeding on this principle we find that the two averages coincide closely, the average of the 8 samples of individuals with double arches being 58.310 and that of the 8 samples of normal individuals 58.303.

Thus it seems evident that our assumption that the vertebra in question has a numerical value of $1^1/_2$ is correct. Hence it follows that it would be just as incorrect to count it as 1 as to reckon it as 2 complete vertebrae. On the other hand we are apparently justified in completely ignoring individuals with double hæmal arch at the 5-last vertebra, provided we are dealing with groups consisting of a large number of individuals.

Our investigation thus shows that vertebrate animals can

realize fractional parts of vertebrae, but it is evident from the table that such individuals are numerically inferior to what we above called "normal" individuals. In reality the former are just as "normal" as the latter. In both cases it is the individual's genetic structure in connection with its environment in the sensitive period, which is deciding the total realized; but it seems as if whole numbers in such organs as vertebrae are more easily realized than fractional parts.

I must here recall that, based on the results of the diallel crossings with trout, I had to distinguish between the two notions personal and generative value (1919, 1 and 1919, 2) and that I found thatan individual's generative vertebral number could have any value whatever between two whole numbers e. g. 58.57 or 62.72 Taking this into consideration the proof that fractions of vertebrae can actually be realized — in other words that also the personal values can be broken numbers — is of interest.

Finally I desire to emphasize that there exist other categories of fused vertebrae than those mentioned here. Thus one of our experiments, which I hope to find an opportunity of discussing later, proves that the vertebral column under the influence of extreme external conditions can develop quite abnormally, with irregular fusions of a greater or a smaller number of vertebrae.

Carlsberg Laboratory, April 15, 1921.

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A PECULIAR MODE OF INHERITANCE AND ITS CYTOLOGICAL EXPLANATION.

BY

O. WINGE.

In Drosophila and other organisms hitherto examined in which sex-linked inheritance is found it is the absolute rule that it has not been possible to show any factor in the unpaired Y-chromosome (or W-chromosome), whereas the X-chromosomes (or Z-chromosomes) contain many factors which are inherited sex-linked.

Goldschmidt[1]), it is true, assumed that a female deciding factor in the Y-chromosome is possibly found in the *Lymantria dispar*, which might serve as an explanation of the appearance of intersexual individuals in his interesting experiments. This, however, is not fully explained, as i. a. the possibility exists that it is a question of plasmatic inheritance. Nor did Seiler succeed in showing any cytological difference in the *Lymantria dispar* as to the male and female chromosomes. — Thus this question may be set aside.

Furthermore Punnett and Onslow[2]) have thought it possible that a factor might be found in the W-chromosome of *Abraxas*, as also Punnett[3]) is inclined to believe that a corresponding factor (for hen-feathering) is found in the fowl.

If we look upon a skeleton-like sketch of the sex-chromosomes e. g. in *Drosophila melanogaster* (fig. 1), we will find that while the X-chromosomes are transferred from generation to generation in zigzag from one sex to the other, the Y-chromosome is

[1]) Untersuchungen über Intersexualität, Zeitschr. f. ind. Abst.- und Vererb.-lehre, Vol. XXIII, 1920, p. 1.

[2]) Onslow, H.: The Inheritance of Wing-Colour in Lepidoptera. V., Journ. of Gen., Vol. 11, 1921, p. 123.

[3]) Genetic Studies in Poultry. III., Journ. of Gen., Vol. 11, 1921, p. 37.

normally bound to the male individuals. Provided the Y-chromosome thus contains a dominant factor, this will come out in a corresponding character, inherited from father to son, generation after generation — or in other words literally inherited sex-limited.

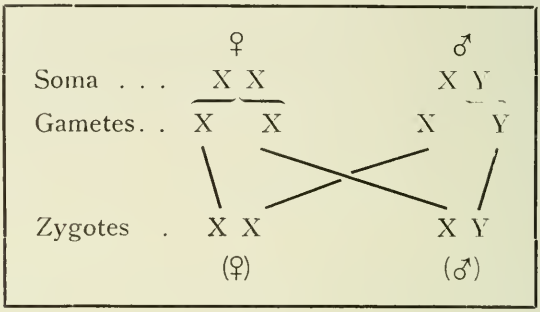

Fig. 1.

As to the hitherto examined cases of so-called sex-limited inheritance, the many experiences to this effect seem to show that the factors which form the basis of the characters in question, do not have their seat in a Y-chromosome (or W-chromosome). The secondary sexual characters are, so far, not inherited sex-limited, but have their seat in chromosomes which are found in individuals of both sexes, although the characters phenotypically only appear in one of the sexes, while they are latent in the other sex.

It would only be the question of a literal sex-limited inheritance, even if it does not allow of definition, if a quality could be shown following the chromosomes, which generation after generation are transferred from father to son, grandson e. t. c. or from mother to daughter, granddaughter e. t. c.

The investigations of the inheritable conditions of the cyprinodont, *Lebistes reticulatus* made on Carlsberg Laboratory, of which Dr. Johs. Schmidt has given a detailed account[1]), have given an exceedingly good instance of this hitherto quite unknown mode of inheritance, and the cytological investigations which I have made in order to explain the phenomenon have now been finished and will be published in this treatise.

[1]) The genetic behaviour of a secondary sexual character. — Comptes-rendus des trav. du Laboratoire Carlsberg, Vol. 14, Nr. 8, 1920.

In order briefly to recapitulate the conditions of inheritance in *Lebistes reticulatus* — as they are accounted for by Dr. Johs. Schmidt in his above mentioned work, it appears that the male and female *Lebistes reticulatus* have a different appearance, the female being considerably larger than the male of a plain greyish green colour, while the male according to the race to which it belongs is adorned in different ways, sometimes with drop-coloured or yellow spots on the side, sometimes with a black spot on the dorsal fin, sometimes with vertical sidestripes, sometimes with a round, sometimes with an elongated caudal fin. The difference in pigmentation and colouring in the males belonging to different races is very great, whereas the females, as mentioned above, virtually are looking alike. If a male with a black spot on the dorsal fin is crossed with a female of a species in which the males have no such spot, all the F_1 male offspring will resemble the father (i. e. have a spot on the dorsal fin), and when attempting to segregate the material into F_2, all the F_2 male will also have a dorsal fin spot. Back-crossing of F_1 males with females of an unspotted form only gives males with dorsal fin spot, whereas F_1 females crossed with males of an unspotted form will get sons quite without dorsal fin spot. In other words it is exclusively the male that decides the looks of his sons while the female does not in any way influence the pigmentation or colouring of her sons. No apparent segregation takes place, but an incessant imitation of the appearance of the father, of course always subject to more insignificant variations which, it is supposed, mainly are of a purely phenotypical nature.

These experiments are carried through to a great extent with about 1500 individuals and in several generations (5—6), so as to give an absolute guarantee as to the results. Furthermore, this holds good not only of a single "race" or "variety", but the same applies to several races of *Lebistes reticulatus*. As the relation between the number of male and female individuals is always about as 1 : 1, there is no reason for supposing the presence of lethal factors, as in some *Drosophila* mutants, causing this mode of inheritance.

It was a natural assumption that we had to do with factors having their seat in chromosomes which were exclusively inherited through the male individuals. It was probable that a disposition in the Y-chromosome was shown here, and in the hopes

of solving the question cytologically I made the necessary fixations of both male and female individuals of the races in question of *Lebistes reticulatus*.

The cytological conditions of *Lebistes reticulatus*.

Lebistes reticulatus is not a pleasant object for an investigation of the chromosome conditions, as the chromosomes are exceedingly small in this fish. On the whole fishes seem to a small degree to have been made the object of experiments. Experience has learned me that the young genital organs both of the male and the female are the organs best adapted for the studying of the chromosomes. Consequently I have mainly examined such organs. The individuals in question of *Lebistes* were decapitated, the genital organs prepared and for some hours fixed in Carnoy's liquid; after the necessary treatment and cutting on the microtome they were stained with Delafield's Hematoxylin.

Spermatogenesis. At an age of three months the males are fertile, provided they have been cultivated under favourable conditions in an aquarium placed in a light room at about 25 degrees. As has been accounted for by Erich Philippi[1]) as regards some kindred species and by Dr. Johs. Schmidt (l. c.) the pairing and fertilisation take place in a very peculiar manner. Through the transformed anal fin (gonopodium) the male during the act of copulation discharges some roundish projectiles ("Spermatozeugmen"), consisting of thousands of spermatozoa, the heads of which form the cap of the projectile, while the tails are lying towards the middle of the ball. The male discharges these "Spermatozeugmen" like shot towards the genital duct of the female. The spermatozoa chemotactically effected by the secretes of the oviduct are set free, and in large quantities they ciliate into the folds of the oviduct in order to fertilise the mature eggs. Several spermatozoa, however, remain in the oviduct and are able to keep alive for several months and when the female a month after the fertilisation has cast her young — generally in a number of 10—50 — these spermatozoa can fertilise the eggs which have

[1]) Fortpflanzungsgeschichte der viviparen Teleosteer *Glaridichthys januarius* etc. Zool. Jahrbücher, Vol. XXVII, Heft 1, 1908.

matured in the meanhwile. During this time the spermatozoa are lying ready by thousands in the folds of the oviduct. After the copulation they are seen lying in the ovary with their heads as near the immature eggs as possible. Off each egg they are seen lying as a shoal, their tails arranged in the form of a convoluted cord, as shown in the microphoto, plate I, fig. 5.

I do not intend to try to give a detailed account of the finer cytological conditions in *Lebistes reticulatus,* but only to point out the most striking features, and besides to account for the peculiar mode of inheritance of the male secondary sexual characters.

In the testis of a fertile male all stages of development are generally found, from the large newly developed primitive mother-cells to spermatocysts with mature spermatozoa. The division of the nucleus generally takes place synchronally in a spermatocyst; however it occurs — especially at a later stage of development — that the contents of a spermatocyst as regards stage of development is divided into two parts of which one part is further divided, whereas the other part remains in a resting stage. Sometimes it is found that there is some difference as to the stage of development in the different spermatogonies of a spermatocyst. During these divisions the chromosomes become very evident; however, it is not possible to ascertain their number, as they are not lying in a single layer, but so close together that it is impossible to count them. At the reduction division in the primary spermatocyte a very clear picture appears, making it possible with absolute certainty to state that the haploid chromosome number is 23. Fig. 1—2 on plate I show two nuclei in which 23 chromosomes may be counted. The chromosomes are placed with great regularity, as 13 chromosomes nearly always are found in the circumference and 10 within the circumference. The chromosomes are very much alike. I myself have partly found, partly induced others to control the existence of this number so many times, that I venture to state with absolute certainty that neither more, nor fewer chromosomes are found in the material which has been at my disposal.

After the reduction division the daughter nuclei are remaining together as seen on plate I, fig. 3, no secondary spermatocytes being formed and are at the same time dividing homoetypically. After this division spermatocytes with 4 nuclear plates (plate I, fig. 4), and afterwards groups of four spermatids may be observed.

A very characteristic feature is the absolute want of stages which may be called synapsis and diakinesis. At most a faint deposit of chromatin may be observed as a compensation for the synapsis.

The spermatids are comparatively quickly transformed into spermatozoa, the nucleus being elongated and forming the arrow formed split head. The spermatozoa quite fill up the cavity of the spermatocyst. Subsequently they gradually move towards the wall of the spermatocyst where they gather in large groups, each having a cell of the wall (Sertoli-cell) of the spermatocyst as a center. On a certain stage the spermatozoa are seen lying towards the wall of the spermatocyst which is formed by one layer of cells, forming a polygonal shoal off each cell in the cellular wall. Gradually the spaces between the polygonal groups are also filled up, all the contents of the spermatocyst contracting into a globular or ellipsoid body, exclusively consisting of spermatozoa whose tails project into the middle of the ball, almost filling it, while the circumference is formed by the heads. The heads are placed very close together, and as generally all the spermatozoa do not succeed in finding a place in the circumference, a new layer of heads — as a second row — is formed. Thus the "Spermatozeugmen" are completed and glide towards the emunctory where they often gather in comparatively great quantities, gradually to be discharged — probably one at a time during the act of copulation.

Oogenesis. On the whole the female *Lebistes reticulatus* become later mature than the males and when fertilised they bring forth a little brood, generally about 5—10. Almost every month they will bring forth another brood, provided the conditions are favourable, as is accounted for in detail in Johs. Schmidt's treatise (l. c.), the number of young gradually increasing; at the same time the females continue to grow considerably for many months.

The oogonies are placed — just as mentioned by Philippi (l. c.) when speaking about *Glaridichthys januarius* — partly one by one in the epithelium of the cavity of the ovary, partly below the epithelium. Here the oocytes are not formed one by one, a whole group being formed by synchronal divisions from a primitive mother-cell, so that often more than half a dozen are

found in the same group. When following these synchronal divisions of the oogonies there is a chance, although a very small one, of finding the stages of the divisions of the nuclei, rendering a counting of the chromosomes possible. Before succeeding in finding some oogonies in the proper stage of division and at the same time cut in the proper position, I have had to undertake a considerable work in vain. A great number was, it is true, observed under division, in most cases at an stage of metaphase, but very often several ovaries may be examined without finding any stages of division at all in the oogonies. It is a matter of course that consequently only a small number of the nuclei under division were placed so favourably that it was possible to count the chromosomes. Sometimes the spindle of the nucleus was cut obliquely and distributed on two sections, which necessarily renders the counting of the chromosomes less accurate, as one or more chromosomes may be cut through or removed by the microtome knive, sometimes the incision plane was not parallel with the plane of division of the nucleus, which also renders the counting difficult.

After many efforts I succeeded in finding some nuclei in the metaphase which seen from the pole were favourably placed. These nuclei appeared to contain 46 chromosomes, i. e. a number corresponding to that of the somatic cells of the male. It must, however, be observed that this absolute number is not given with as great certainty as that with which the number 23 is given as regards the genital cells of the male. Only in a single one 46 chromosomes were counted with sufficient certainty (plate I, fig. 6). Fig. 7 on plate I seems to show only 45 chromosomes. It is quite out of the question that a higher number than 46 may be found.

As furthermore 23 pair of gemini are found in diakinesis (plate I, fig. 8) there can be no doubt, that also the female has 46 chromosomes.

The further development of the oogonies is of no interest for our object. The oocytes which are finally formed are growing considerably, a great yolk being formed. Not until the fertilisation it is supposed that the reduction division, the egestion of the polar bodies, takes place, although I have not succeeded in finding this stage represented in the material at my disposal. The egg with the embryo remains in the follicle until it is mature and the birth takes place.

During a lapse of time of about 20 minutes to an hour and a half all the young have usually been bred and are often still provided with so much yolk that they have sufficient food for some days. Besides they immediately start snatching small particles in the water.

As 46 chromosomes are found in the metaphase in the nuclei of the somatic cells of the female and 23 pair of gemini in the diakinesis, the number of chromosomes in the two sexes is the same. No difference is to be found between the sex chromosomes and the autosomes.

Conformity between the mode of inheritance and the chromosome conditions.

As the number of chromosomes in the two sexes is the same, it may without doubt be supposed that the chromosome conditions so far are corresponding to those found in *Drosophila*, the total number of chromosomes, however, being considerably larger. The result of the fertilisation is seen from the following sketch (fig. 2).

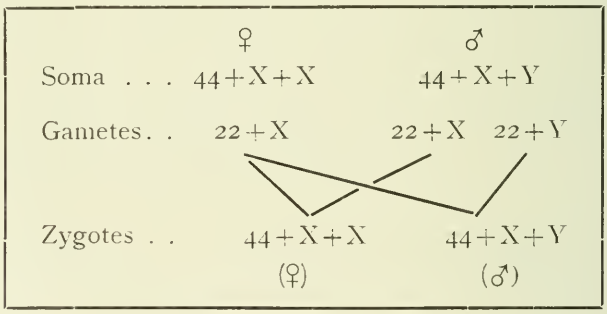

Fig. 2.

The Y-chromosome is, as will be seen, connected solely with the male individuals, and the factors this chromosome contain will not be found in the female individuals, but in all the young of male sex, and be visible in case none of the factors in the autosomes predominate over the factors inherited through the Y-chromosome. It seems that the factors of the Y-chromosome fully dominate those of the autosomes, for, as already stated, the young of male sex always resemble the father so much that there seems to be no Mendelian segregation whatever which has influence upon their design and colouring.

This mode of inheritance has not yet been observed elsewhere, as inheritable characters, exclusively inherited through the male line, have not as yet — as mentioned above — been shown elsewhere.

The ideas "sex-limited" and "six-linked" inheritance will from now on be more difficult to separate. According to Morgan's definition the sex-linked characters depend on such factors as have their seat in the sex chromosomes. Our case belongs to this group. On the other hand one cannot think of characters being inherited sex-limited to a greater extent than the secondary sexual characters in *Lebistes* which just have been mentioned. I propose the indication onesided inheritance for our case — especially onesided masculine inheritance. This indication should then be used for the factors which have their seat in the sex chromosomes only appearing in the one sex (Y- or W-chromosomes), whereas sex-linked inheritance concerns such factors as have their seat in the sex chromosomes found in both sexes (X- or Z-chromosomes). Sex-limited inheritance should probably, even though the indication is very unsatisfactory, continue to be employed as regards the factors for the secondary sexual characters found in the autosomes.

Whether the Y-chromosome itself also is sexdeciding, is uncertain. Experiences gained from the *Drosophila* researches have proved that one must indeed take care not to state anything certain on this subject. Only the appearance of *Lebistes* individuals with differing chromosome conditions will probably be able to decide this question.

Finally should be only briefly stated that on the Carlsberg Laboratory 2 forms of *Lebistes* are cultivated which in regard to inheritance of the secondary sexual characters of the male differs from the other "races". The result of the experiment with these forms will be published in a following treatise, as these experiments have not as yet been finished; but so much is known for certain, that it is a question both of onesided masculine inheritance as also of sex-linked inheritance. And the demonstration of both modes of inheritance in *Lebistes* does to a high extent support my view given in this treatise as to the chromosome conditions of this form.

Carlsberg Laboratory, Copenhagen, December 1, 1921.

Explanation to Plate I.

Fig. 1, 2, 3, 4, 6 and 7 were drawn with the aid of camera, using Zeiss' homog. imm. 1,5 mm. and comp. oc. 18. By reproduction they are reduced to $^2/_3$.

Fig. 5 is on unretouched microphotograph.

Fig. 8 was drawn with the aid of camera, using Zeiss' homog. imm. 2 mm. and comp. oc. 18. By reproduction it is reduced to $^1/_2$.

Fig. 1-2. Primary spermatocytes in heterotypic metaphase. 23 chromosomes.

3. Spermatocyte in homoeotypic division. No formation of secondary spermatocytes.

4. Anaphase of homoeotypic division.

5. A shoal of spermatozoa off an egg in a fold of the ovary.

6-7. Nuclear plates from oogonia, showing resp. 46 and apparently only 45 chromosomes.

8 a, b, c. Diakinesis in nucleus of a primary oocyte. 3 sections. 23 pairs of gemini.

1

2

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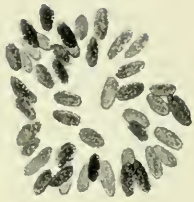
4



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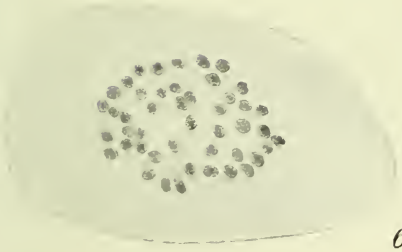

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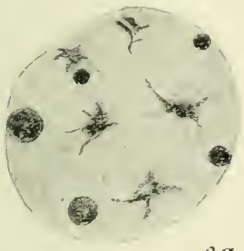

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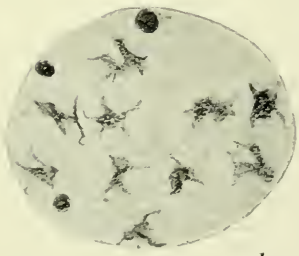

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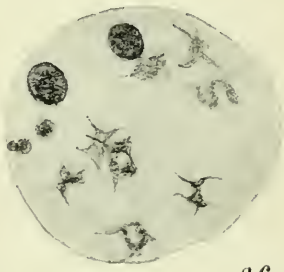

8c

Ö. Winge: On the Cytology of *Lebistes reticulatus*.

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ONE-SIDED MASCULINE AND SEX-LINKED INHERITANCE IN *LEBISTES RETICULATUS*.

BY

Ö. WINGE.

From Schmidt's work (1920) on the inheritance of secondary male sex-characters in *Lebistes* we meet with the peculiar condition of an apparent absence of segregation for such characters; for all male offspring, notwithstanding the origin of the mother, always present the same appearance as the male parent[1]).

It was impossible in any of the races mentioned in Schmidt's work to ascertain any influence whatever of the mother on the secondary sex-characters of the male offspring. This, however, did not exclude the possibility that other races behaved otherwise.

The one-sided mode of inheritance might be supposed to depend on a male sex-chromosome — as the Y-chromosome of *Drosophila* — constantly being transferred from father to son, without ever entering the female individuals, and that this sex-chromosome contained the factors for the said secondary male sex-characters.

This supposition induced me to make a cytological investigation of *Lebistes*. It appeared that both the male and the female had the same number of chromosomes, i. e. 46 in the somatic cells and 23 in the sexual cells; consequently the number of chromosomes of the female is probably 44 + X + X, while that of the male is 44 + X + Y.

However, I have not succeeded in laying down any morphological or other differences in the chromosomes, which render it

[1]) See also my previous treatise in this journal, Vol. 14, N° 17, 1922.

possible to distinguish the sex-chromosomes from the autosomes. Thus it was a mere hypothesis to suppose that both the Y-chromosomes and the X-chromosomes existed in *Lebistes*, so that further proofs were demanded, to make out the correctness of this hypothesis.

Although a morphologically traceable Y-chromosome is not found, I was on the other hand obliged to take it for granted that the only explanation of the one-sided male inheritance was that such a Y-chromosome existed, and that it was the bearer of the one-sided inherited factors. The very fact that it was a question of male inheritance must be said to support the supposition to a far higher degree than a one-sided female inheritance, for in the latter case it was necessary to reckon with the possibility of plasmatic inheritance. As is well known, several cases of inheritance have been shown within the vegetable kingdom, which it seems possible to explain only by the supposition that in some cases plastids transfer the inheritable characters, while in other cases this is done by cytoplasm (see in this respect my treatise of 1919).

One-sided female inheritance could not with the same obviousness be indicative of the existence of a W-chromosome. It was necessary to take into consideration the possibility of cytoplasmic inheritance. The female cytoplasm, it is true, is transferred to both sexes; thus a one-sided female inheritance through the cytoplasm might, on a superficial view, seem out of the question. But where at the same time it was a question of female sex-limited inheritance — i. e. provided only female individuals phenotypically showed the said characters — as only the males of the *Lebistes* phenotypically show the inherited secondary sex-characters — it might, in spite of everything that might be indicative that a female W-chromosome was the bearer of the said factors, virtually be a question of a cytoplasmic inheritance, that acts like a one-sided female inheritance. — It is true, that this is mere supposition, but there would appear to be no strong objection against it.

As mentioned in my last work, Punnett, Onslow and Goldschmidt thought it necessary to seek the explanation of certain conditions of inheritance in the presence of factors in the corresponding W-chromosome, i. e. what I call one-sided female inheritance, but it has not yet been shown with certainty whether

the supposition is correct. Although I do not doubt for a single moment that a one-sided inheritance transferred through the W-chromosome must be found, I have desired in the foregoing to point out the difficulties in distinguishing the one-sided female inheritance through the chromosomes from the cytoplasmic one.

But although the existence of a Y-chromosome in *Lebistes* thus on account of the conditions of inheritance, seems to be almost certain, it would of course be a great support for the correctness of the supposition, if it were possible to show the existence also of the X-chromosomes. For if the diploid chromosome number is 46 both in the male and in the female, and if a Y-chromosome is found, X-chromosomes must also be found and thus a sex-linked inheritance is taking place, provided these X-chromosomes contain factors.

The present paper will prove that such X-chromosomes occur.

On theoretical considerations Castle (1909) long ago advanced the idea that secondary male sex-characters might accompany the Y-chromosome, a supposition of which the correctness has hitherto not been proved in other organisms.

In *Drosophila melanogaster* the Y-chromosome is empty, although it must be supposed to be of certain importance, as the males produced by "non-disjunction" are sterile if the Y-chromosome is wanting. One might say that the Y-chromosome in this case contains a "normal factor" necessary to the fertility of the males, so that this factor may be contrasted with the recessive, sex-linked, lethal factors, that have a killing effect on the males.

Although the Y-chromosome of *Drosophila* evidently has a physiological effect, it does not contain any factors in the sense that the males are morphologically marked hereby, as is the case with many of the other factors. And as on the whole no allelomorph to this possible "normal factor" has been shown in the Y-chromosome of *Drosophila*, it is only in a very improper sense that a factor is found. The factor content of the Y-chromosome in *Drosophila* must in reality be said to be of a doubtful nature.

In *Lebistes* on the other hand different values of the Y-chromosome have, as we know, been shown, since the Y-chromosome of each race has its special effect; and this brings it

about that the secondary male sex characters of each single race are inherited through the male line.

As yet it has not been possible to state whether the Y-factors in the different races are placed in the same locus in the chromosome, i. e. whether they are allelomorphs. In case individuals with 2 Y-chromosomes were found, crossing-over might perhaps be possible, thereby proving whether they were absolutely linked real allelomorphs. Nor is it possible to say whether the *Lebistes* factors are dominant or recessive; for whether they are one or other their effect will presumably appear as long as no corresponding Y-chromosome is found. Their effect is so far epistatic in that no factors have as yet been found in the autosomes or the X-chromosomes that might conceal their effect.

As before mentioned, it is characteristic that the X-chromosome and the Y-chromosome in the spermatocytes during the reduction division do not act otherwise than the autosomes, and cannot be distinguished from the latter; I therefore take it for granted that the X-chromosome of the male individual in all respect acts like the partner of the Y-chromosome. That crossing-over between these two chromosomes may even take place — a phenomenon of extreme interest — will be treated in the last section of this treatise, although I reserve myself to undertake more elaborate investigations in a later work.

After these more general observations I may pass on to submit the special part of my investigations.

Effects of the Factors in X- and Y-chromosomes.

In order to facilitate the understanding of the crossing experiments undertaken by me, I shall submit the main results of these experiments at once; afterwards I shall mention the experiments themselves which form the basis of the results.

As mentioned before, *Lebistes reticulatus* has 46 chromosomes in the somatic cells of both sexes, the female having $44 + X + X$, while the male has $44 + X + Y$. No attention will be paid to the 44 autosomes here, for it is only the sex chromosomes that play an essential part in the inheritance of the secondary male characters, i. e. only the sex chromosomes contain the essential factors for the colour patterns, which are here taken into consideration.

In the different *Lebistes* races the X-chromosomes, as also the Y-chromosomes, are of different value, i. e. contain different characteristic colour factors. The Y-chromosome always contains factors for some colour pattern or other, while the X-chromosomes only in certain races contain such factors, being empty in other races.

Where only the Y-chromosome in a given race contains factors the above-mentioned one-sided male inheritance will be found, dependent on the Y-chromosome being inherited only through the male line. If on the other hand the X-chromosomes also contain colour factors, the mode of inheritance on crossing is found to be a combination of the usual sex-linked inheritance and one-sided masculine inheritance.

Even if female individuals contain colour factors in their X-chromosomes, they remain phenotypically uncoloured, as only the males are coloured. This recalls de Meijere's account of *Papilio Memnon* (1910), and Fryer's case of *Papilio polytes* (1913), in both of which the males always have the same appearance, while the females may be of different phenotype according to their genotypical content. That it is the opposite sex, the females, which in *Lebistes* are always phenotypically alike, is evidently attributable to the fact that it is the female which here is the homogametic sex, while it is the male in the butterflies.

The two X-chromosomes of a *Lebistes* female may be alike or may differ as to their factor content. Thus we have homozygotic and heterozygotic females; the latter form two kinds or eggs in equal number.

A male *Lebistes* is always heterozygotic, as it is heterogametic, its X- and Y-chromosome not containing the same factors. Thus the male *Lebistes* constantly forms two kinds of spermatozoa, of which one — the one with Y — is male determining, while the other — with X — is female determining.

In the male *Lebistes* containing a colour factor both in the X-chromosome, and in the Y-chromosome, the corresponding characters are phenotypically visible at the same time. The effects of the two factors are combined, leading to a sort of compromise between them. Thus it is possible in each male individual to see what factors it contains, whereas the females phenotypically are indistinguishable.

As only the males phenotypically show the characters, we

have sex-limited inheritance as a further complication; this badly designated conception is, as far as inheritance is concerned, of minor importance, as it is only a question of phenotypical differences between the male and female.

In Plate 2 at foot, 6th to 8th row I have in skeletonlike form reproduced the single effects of the factors[1]) found in the *Lebistes* races with which I am engaged in this work, as also some instances of the combining effects of the same factors.

We shall here go over the said factors, or — more correctly — the "effects of the chromosomes" on the compound characters. They are called X and Y according as the factors are contained in an X- or Y-chromosome, and as index a letter is added to indicate in shortened form the most characteristic colour effect of the factor.

Of the X-chromosomes only two will be mentioned:

X_o — does not involve any colour pattern of the males (see Plate 2).

X_s — *(sulfureus)* (see Plate 2) involves:

1) sulphur yellow colour in the dorsal fin and a dark dot that only at times is visible in the said fin.
2) sulphur yellow colour in the tail and in the caudal fin.
3) Red colour in the lower edge of the caudal fin.

Of the Y-chromosomes we shall mention 4 types:

Y_r — *(ruber)* involves (see Plate 2):

1) Red colour proximally in the upper edge of the caudal fin.
2) Large oblong red side spot, lying for the most part below and behind the dorsal fin.
3) Dark side dot in the tail at the base of the caudal fin.

Y_i — *(iridescens)* involves (see Plate 2):

1) A characteristic mother-of-pearl sheen on the body.
2) 2-3 red smaller side spots.
3) Black side dot on the tail near the caudal fin.
4) Black side dot on the body.
5) Red colour proximally in the middle part of the caudal fin.

Y_m — *(maculatus)* involves (see Plate 2).

1) Large black spot in the dorsal fin.

---

[1]) That they are not single factors but complex ones is a matter which will be dealt with later.

2) Large red side spot below and in front of the dorsal fin.
3) Black dot at the gat (sometimes invisible).

Y_f — *(ferrugineus)* involves (see Plate 2):

1) Black rust-coloured part in the proximal part of the caudal fin.
2) Black side dot in the tail near the caudal fin.

The said effects of the factors must be understood in the sense that I have only mentioned the constant "positive" effects. I have for example stated that Y_m involves a black spot in the dorsal fin, while it is not stated — what in itself might be justified — that Y_i involves the uncoloured dorsal fin. Besides the said colour patterns, dealt with above, other less conspicuous differences might be included. Thus, there are cases in which a little red side-spot occurs more or less constantly on the front of the breast, and so on. Doubtless the phenotypical variation plays a part as regards these less constant patterns, but it is not impossible that the autosomes may contain important factors. We need not busy ourselves with this question here. The colour patterns beyond the said characteristics, are under all circumstances of such small extent that they do not in any way conceal the factor effects described.

The races of *Lebistes* that have been used for the crossing experiments mentioned in this treatise have the following formulae:

	♀	♂
The "Magdeburg Race" (Plate 2, upper row)	X_sX_s	X_sY_r
The "Spot Race" (Plate 2, 3rd row to the right)	X_oX_o	X_oY_m
"The old Race" (Plate 1, 5th row to the right)	X_oX_o	X_oY_i

The names of the races refer to the designations which they have received during the daily work in the Laboratory. When comparing the pictures of the coloured plates and the above descriptions of the effects of the factors, one will easily obtain a notion of the pecularities of the single races.

In addition to the above mentioned three races, the effect

of the Y-chromosome has been described above in a fourth race, i. e. the Y_f-chromosome of the so-called "Hamburg Race". As the conditions of inheritance of this race have not as yet been used in the experiments treated here, I shall confine myself to the above mention of the factor effect of the Y_f-chromosome and to the illustration on Plate 2.

The Crossing Experiments.

As will be seen from the preceding, it only holds good of a single one of the above-mentioned races — the "Magdeburg Race" — that the X-chromosomes (X_s) contain a factor for the colour pattern of the males, i. e. the factor (the complex factor) s. In the "Spot-race" and the "Old Race" on the other hand the X-chromosomes (X_o) are empty, as is also the case with the Y-chromosomes of *Drosophila*.

Although, I have determined the presence of colour factors in X for other *Lebistes* races, the work is not yet sufficiently complete for publication; and, therefore, I shall confine myself to the crosses I have made with the "Magdeburg Race" in order to show the combined sex-linked and one-sided masculine inheritance.

A general description of the "Magdeburg Race" is as follows:

The female individuals, as with all the *Lebistes* forms, are dark greyish green, semi-transparent, without any colour pattern, and with nearly colourless fins. The males are characteristic in the body and the tail having a vivid red and yellow pattern. Especially conspicuous is a large oblong red side-spot below the dorsal fin and extending somewhat behind it. On the breast may be found a little black and a little red dot, the black one usually above the red one. The dorsal fin is of a vivid yellow colour and sometimes also has a little black dot. The lower edge of the tail has the same vivid yellow colour as the dorsal fin, and there is a dark side dot on the tail near the caudal fin. The form of the caudal fin is not quite round but has a tendency to be prolonged above and below, the prolongation however not being flagelliform. The prolongation tendency often shows itself in the hindmost edge becoming more or less vertical instead of rounded. The caudal fin has a red and yellow pattern,

of which a vivid red patch at the top proceeding from the root of the fin, is especially noticeable, while another somewhat similar patch is seen below.

It has already been stated, that the factors for the colour pattern of the "Magdeburg Race" are placed partly in the X-chromosome, partly in the Y-chromosome. I would refer here to the illustration of the three "Magdeburg" males in the first row of Plate 2 and to the figures in the 6th row on the same Plate representing the single effect of Y_r and X_s as also the combined effect of $Y_r + X_s$, which thus is a skeletonlike produced "Magdeburg" male.

The crossing experiments I have made with the "Magdeburg Race" were commenced as early as 1917, but as other work was then considered to be of greater importance, the recording and drawing of the different types of the young individuals were partly neglected. During the last year I have again thoroughly gone into the material, so that I have realized that beside the one-sided masculine inheritance, sex-linked inheritance is also taking place. This is so much more to be regretted, as the *Lebistes* females of late years have suffered from a rather limited propagation ability, so that only rarely have I had such large hatches as in former years. 20 youngs in one hatch is at present highly satisfactory, while double the number in former years was general. The birth-rate has also decreased, as the females now seldom bring forth one hatch of young per month as was formerly the case.

The crossings made are the following:

I. "Spot Race" ♀ 140 × "Magdeburg Race" ♂ 114

	X_oX_o	X_sY_r
	♀♀	♂♂
F_1 Theoretically:	All X_oX_s	All X_oY_r
Found:	[Of which	44 X_oY_r All[1]) as in
	♀ 174 used in exp. IV	Plate 2, 2nd row.
	♀ 175 — — V	[Of which some
	[♀ 176 — — VI]	[used forexp. XIV.]

[1]) Besides a deviating male, the only individual in all the experiments mentioned in this treatise that did not fall in with what was expected. It will be made the object of a closer examination later.

	♀♀	♂♂
F_2 Theoretically:	$1X_oX_o : 1X_oX_s$	$1X_oY_r : 1X_sY_r$
Found:		$7X_oY_r + 7X_sY_r$

II. "Magdeburg" ♀ 244 × "Spot Race" ♂ 189

	X_sX_s	X_oY_m
	♀♀	♂♂
F_1 Theoretically:	All X_sX_o	All X_sY_m
Found:		1 [1]) X_sY_m depicted in Plate 1, at top to the right. [Used as ♂ 334 in exper. XIII.]

III. "Magdeburg" ♀ 188 × "Spot Race" ♂ 189

	X_sX_s	X_oY_m
	♀♀	♂♂
F_1 Theoretically:	All X_sX_o	All X_sY_m
Found:	[Of which ♀ 258 used for F_2 ♀ 335 — · exp. VII ♀ 337 — · — VIII Others — · — IX]	21) X_sY_m [Used as ♂ 259 in exp. XI, XII and for F_2 ♂ 280 in exp. X.]

	♀♀	♂♂
F_2 Theoretically:	$1X_sX_s : 1X_oX_s$	$1X_sY_m : 1X_oY_m$
Found: [of F_1 ♀ 258 × F_1 ♂ 259]		$3X_sY_m + 1X_oY_m$

The above-mentioned F_1 individuals have — as will be seen — the following formulae:

All F_1 ♀♀: ..... X_oX_s
F_1 ♂♂ from "Spot Race" × "Magdeburg": X_oY_r
— · "Magdeburg" × "Spot Race": X_sY_m

The appearance of the above-mentioned 47 F_1 males agrees with what was theoretically expected, as they all have received the factors characteristic for the Y-chromosome of the paternal race. That the nature of the offspring from the F_1 parents of both sexes is consistent with the theory, will appear partly from

[1]) Several individuals of the same appearance have not been entered.

the appearance of the F_2 individuals, and partly from the following crossings.

As will be seen, the two types of F_1 males which are produced by the reciprocal crosses contain quite different colour factors, namely X_oY_r and X_sY_m respectively, while the F_1 females all have the formula X_oX_s.

Re-crossing of F_1 ♀♀ X_sX_o with the "Magdeburg"-father X_sY_r.

IV. ♀ 174 [from exp. I] × ♂ 114

	X_sX_o	X_sY_r
	♀♀	♂♂
Theoretically:	$1X_sX_s : 1X_oX_s$	$1X_sY_r : 1X_oY_r$
Found:		$4X_sY_r + 4X_oY_r$

V. ♀ 175 [from exp. I] × ♂ 114

	X_sX_o	X_sY_r
	♀♀	♂♂
Theoretically:	$1X_sX_s : 1X_oX_s$	$1X_sY_r : 1X_oY_r$
Found:		$1X_sY_r + 1X_oY_r$

VI. ♀ 176 [from exp. I] × ♂ 114

	X_sX_o	X_sY_r
	♀♀	♂♂
Theoretically:	$1X_sX_s : 1X_oX_s$	$1X_sY_r : 1X_oY_r$
Found:		$2X_sY_r + 2X_oY_r$

The three F_1 females (experiment IV-VI) thus by re-crossing with the father have given two kinds of sons in equal number, in all 7 X_sY_r: 7 X_oY_r as expected. Despite the very small number an equal number of the two types has by chance fallen in each of the three crossings.

Other Crossing Experiments with F_1-♀♀ X_sX_o.

VII. ♀ 335 [from exp. III] × "Old Race" ♂ 336

	X_sX_o	X_oY_i
	♀♀	♂♂
Theoretically:	$1X_sX_o : 1X_oX_o$	$1X_sY_i : 1X_oY_i$
Found:		$7X_sY_i + 5X_oY_i$

Individuals from this cross will be found figured on the lower half of Plate 1, where female 335 and male 336 are seen reproduced with 9[1]) of the sons, viz. 5 with X_sY_1 and 4 with X_oY_i.

VIII. ♀ 337 [from exp. III] × "Spot Race" ♂ 338

	X_sX_o	X_oY_m
	♀♀	♂♂
Theoretically:	$1X_sX_o : 1X_oX_o$	$1X_sY_m : 1X_oY_m$
Found:		$9X_sY_m + 5X_oY_m$ [Of which ♂379 X_oY_m used for exp. XVIII.]

Individuals from this cross will be found on Plate 2, rows 3—5. Female 337 and male 338 are figured in the third row, while 3 sons with X_sY_m and 3 with X_oY_m are found in the fourth and fifth rows.

IX. F_1 ♀♀ [several individuals of exp. III] × "Old Race" ♂ 340

	X_sX_o	X_oY_i
	♀♀	♂♂
Theoretically:	$1X_sX_o : 1X_oX_o$	$1X_sY_1 : 1X_oY_1$
Found:		$11X_sY_i + 14X_oY_i$

It will be seen from the above experiments that F_1 females ex "Magdeburg" × "Spot Race" together with the reciprocal accord with the theory in having the formula X_sX_o; for half of their male offspring show yellow in the dorsal fin, on the lower edge of the tail, and in the caudal fin, together with red on the lower edge of the caudal, all of which is due to the chromosome X_s, while the other half lack these colours, as their X-chromosome, X_o, does not contain any colour factor. In accordance with the theoretically expected relation 1 : 1, we find that the off-spring of F_1 females, X_oX_s in the above-mentioned experiments were 39 males with X_o and 44 with X_s. When we include the off-spring of the X_oX_s females mentioned below, the theore-

[1]) On account of lack of space only a few individuals from the different experiments are figured.

tical expectation of equality is exactly attained, as altogether we have 50 individuals with X_o and 50 with X_s.

Crossing experiments with F_1 ♂♂ X_sY_m (from "Magdeburg" × "Spot Race").

X. "Spot Race" ♀ 279 × ♂ 280 [from exp. III]

	X_oX_o	X_sY_m
	♀♀	♂♂
Theoretically:	All X_oX_s	All X_oY_m
Found:		11 X_oY_m

XI. "Spot Race" ♀ 260 × ♂ 259 [from exp. III]

	X_oX_o	X_sY_m
	♀♀	♂♂
Theoretically:	All X_oX_s	All X_oY_m
Found:		13 X_oY_m

XII. "Spot Race" ♀ 341 × ♂ 259 [from exp. III]

	X_oX_o	X_sY_m
	♀♀	♂♂
Theoretically:	All X_oX_s	All X_oY_m
Found:	[Of which ♀ 353 used in exp. XVII]	3 X_oY_m [Of which used ♂ 364 in exp. XIX ♂ 365 - - XX]

XIII. "Spot Race" ♀ 333 × ♂ 334 [from exp. II]

	X_oX_o	X_sY_m
	♀♀	♂♂
Theoretically:	All X_oX_s	All X_oY_m
Found:	[Of which used ♀ 350 in exp. XV ♀ 341 - - XVI]	36 X_oY_m

(See moreover experiment III F_2).

Individuals from this experiment are reproduced at the top of Plate 1. In the first row female 333 and male 334 are seen, and in the following three rows 9 out of the 36 sons of similar appearance. These all have the character of the pure "Spot

Race" (X_oY_m), as the sex-linked inheritance involves that none of the sons receives the X_s-character, while this character on the other hand is transferred cryptomerically to all the daughters.

Experiments X-XIII show very clearly the combined sex-linked and one-sided masculine inheritance in *Lebistes*. While none of the F_1 males used (X_sY_m) transfer the X_s-colours of the "Magdeburg Race" to their sons — 63 in number — all the sons have received the one-sided masculine inherited Y_m chromosome and consequently the black spot in the dorsal fin and so on.

Crossing Experiments with F_1 ♂♂ X_oY_r (from "Spot Race" × "Magdeburg Race").

XIV. "Spot Race" ♀ 200 × Several F_1 ♂♂ [from exp. I]

	X_oX_o	X_oY_r
	♀♀	♂♂
Theoretically:	All X_oX_o	All X_oY_r
Found:		$8X_oY_r$

Crossing Experiments with segregated X_oX_s ♀♀.

XV. ♀ 350 [from exp. XIII] × "Spot Race" ♂ 354

	X_oX_s	X_oY_m
	♀♀	♂♂
Theoretically:	$1X_oX_o : 1X_sX_o$	$1X_oY_m : 1X_sY_m$
Found:		$1X_oY_m$

XVI. ♀ 351 [from exp. XIII] × "Spot Race" ♂ 355

	X_oX_s	X_oY_m
	♀♀	♂♂
Theoretically:	$1X_oX_o : 1X_sX_o$	$1X_oY_m : 1X_sY_m$
Found:		$10X_oY_m + 5X_sY_m$

XVII. ♀ 453 [from exp. XII] × "Spot Race" ♂ 357

	X_oX_s	X_oY_m
	♀♀	♂♂
Theoretically:	$1X_oX_o : 1X_sX_o$	$1X_oY_m : 1X_sY_m$
Found:		$1X_sY_m$

Crossing Experiments with segregated X_oY_m ♂♂.

XVIII. "Spot Race" ♀ 377 × ♂ 379 [from exp. VIII]

	X_oX_o	X_oY_m
	♀♀	♂♂
Theoretically:	All X_oX_o	All X_oY_m
Found:		$6X_oY_m$

XIX. "Spot Race" ♀ 360 × ♂ 364 [from exp. XII]

	X_oX_o	X_oY_m
	♀♀	♂♂
Theoretically:	All X_oX_o	All X_oY_m
Found:		$3X_oY_m$

XX. "Spot Race" ♀ 361 × ♂ 365 [from exp. XII]

	X_oX_o	X_oY_m
	♀♀	♂♂
Theoretically:	All X_oX_o	All X_oY_m
Found:		$2X_oY_m$

Finally male 383 which originated in experiment IV, and which, from its appearance, had the formula X_sY_r was crossed with a female of another race. The 6 males thus produced all had the red colour pattern proximally in the upper edge of the caudal fin and the other peculiarities accompanying Y_r, while they all lacked the red and yellow patterns accompanying X_s, as was expected.

Crossing-over between the X- and Y-chromosome.

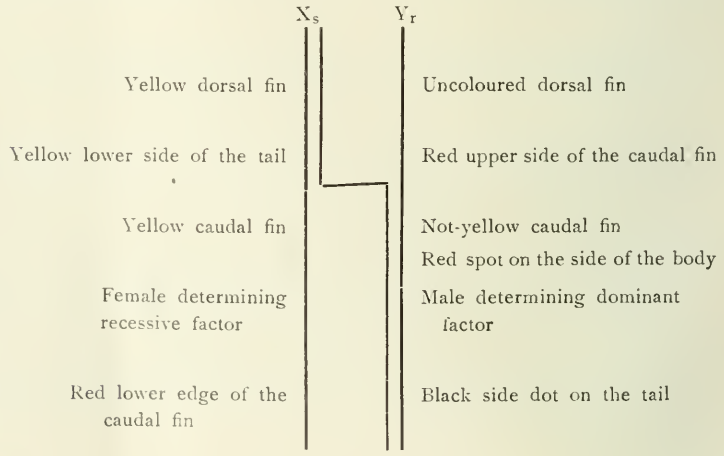
From the above experiments we cannot decide whether each single colour character depends on one, or more genes As long as the chromosomes retain their integrity, it is impossible to decide whether a whole series of factors in the chromosome, or only a single one, is responsible for the hereditary phenomena.

However, I have in my experiments a few highly interesting cases which admit of no doubt that the colour characters in a chromosome are due to more than a single gene, since I have substantiated crossing-over between the X- and Y-chromosome in *Lebistes* males.

In experiment I it was found that when crossing a pure "Spot Race" female (X_oX_o) with a pure "Magdeburg" male (X_sY_r) 44 males were produced, all of which looked alike (c. f. Plate 2, second row). Characteristic of all these F_1 males (X_oY_r) was 1) a red colour proximally in the upper edge of the caudal fin, 2) a long red side spot placed below and behind the dorsal fin, 3) a dark side dot in the tail near the caudal fin, sometimes displaced into the caudal fin, and 4) a colourless dorsal fin, i. e. they all showed the characters due to the factors in the Y-chromosome. None of them had the yellow colour on the dorsal fin, on the lower side of the tail and on the caudal fin, nor the red colour on the lower edge of the caudal fin, which accompanies the X_s-chromosome of the "Magdeburg-Race". — All this is in accordance with the fact that the X-chromosome alone was transferred to all the daughters of male 114 (X_oX_s).

In addition to the 44 males another male individual was, however, found (mentioned in foot-note p. 9) which was of quite special interest, as it had the yellow dorsal fin and the yellow colour on the lower side of the tail which follows the X_s-chromosome, while it lacked completely the red and yellow colours in the caudal fin, which was consequently uncoloured.

As I have found a somewhat similar instance in another of my cultures, I entertain the view that the said individual is brought about by crossing-over between the X_s- and the Y_r-chromosome, as shown diagramatically in the following sketch of the chromosomes:



This is the only way in which the origin of the individual can be explained.

I am sorry to say that the individual in question was not submitted to any genetic analysis, for it occurred at such an early stage of the experiments — being born in October 1917 — that I did not fully realize its significance at that time, as I was engaged in other investigations. Therefore, the individual was only registered, while I myself drew it with colours in June 1918 together with its 44 brothers. On the drawing of the individual, which I recollect very well, I noted: "Very anomalous specimen, notwithstanding fully developed."

In itself it is not strange that a crossing-over may take place between chromosomes, which are morphologically alike, and which evidently conjugate quite as the autosomes previous to the reduction division, and perhaps it is correct to suppose that, at any rate here in *Lebistes*, there is no other principal difference between the X- and Y-chromosomes than that the latter contains a dominant factor for the male sex, while the former contains the allelomorphic factor for the female sex.

The case is of considerable interest, as it suggest that a crossing-over between an X- and a Y-chromosome may take place, and the hitherto sharp limit between the X- and Y-chromosomes has been done away with. I suppose that all investigators on heredity will realize the highly important consequences in respect of the theory on heredity entailed by this fact.

I expect before long to be able to give the decisive proof of the crossing-over by the experiments I am now undertaking, experiments which already are fairly advanced.

Discussion.

As will appear from the experiments, the mode of inheritance of the secondary sex-characters in *Lebistes* is clearly either one-sided masculine, namely when the X-chromosomes are empty, or a combination of one-sided masculine and sex-linked inheritance, viz. when the X-chromosome contains colour factors. To this must be added that the whole mode of inheritance is sex-limited to the male individuals, as the factors are found cryptomerically in the females.

In none of the races hitherto examined by me, were Y-chromosomes found without colour factors; therefore it is the opposite of the condition in *Drosophila*, where it is the Y-chromosome that lacks factors affecting the morphological structure.

Since an exchange between X- and Y-chromosomes, as briefly pointed out, may take place, it is probable only a matter of time when it will be possible to find in Nature — or to produce experimentally — *Lebistes* males without colour factors in the Y-chromosome. There is a possibility that the sex-determining factor is extremely closely linked to some colour factor or other, so that crossing-over only takes place very seldom. That the sex determining factors should be identical with some of the colour factors is, it is true, improbable, as males of different *Lebistes*-races have different colour factors, but notwithstanding they all evidently contain a dominant male sex factor. But as regards the sex factors it may also be a question of multiple allelomorphs.

The allelomorph conditions are as yet an open question as far as *Lebistes* is concerned. Although the factors for colour pattern or want hereof found in the X-chromosomes act like allelomorphs in the above-mentioned experiments, it is certainly only on account of the relative rareness of the crossings-over or of the insufficiency of the material that this appears to be the case. At any rate the above-mentioned cross-over male has shown that the X_s-character and the Y_r-character are divisible unities.

I cannot finish this work without conveying my warmest thanks to Dr. Johs. Schmidt, Director of the Physiological Department of the Carlsberg Laboratory, who entrusted me with the *Lebistes* material for further investigation, when I left the Laboratory in spring of 1921. He has also provided room for the *Lebistes* cultures, until my own Laboratory is able to contain the necessary aquaria. I also thank Mr. J. Clausen, M. A. who with great care has assisted me of late years, and the commissionaire of the Laboratory Mr. N. R. Poulsen, who has had charge of the feeding of the fishes.

Summary.

1. It is shown that in a race of *Lebistes reticulatus* sex-linked inheritance of the secondary male sex-characters occurs in connection with the one-sided masculine inheritance found in *Lebistes*-races hitherto examined.

2. This result agrees with the previous demonstration of 46 chromosomes in both sexes of *Lebistes*, the female having presumably $44 + X + X$ chromosomes, and the male $44 + X + Y$. The factors found in the X-chromosomes show sex-linked inheritance, these in the Y-chromosomes show one-sided masculine inheritance.

3. Two kinds of X-chromosomes are described differing as to the genetic content, of which one is empty (X_o), while the other (X_s) contains characteristic factors for secondary male sex-characters. The colour effects of the chromosomes are depicted and described.

4. Four Y-chromosomes differing as to their genetic content are described (Y_r, Y_i, Y_m, Y_f) that severally contain characteristic factors for secondary male sex-characters. The colour effect of the four Y-chromosomes are described and depicted.

5. If both the X- and Y-chromosome in a *Lebistes* male involve factors for colour pattern, the colour effects in the individual are added up; consequently it is possible by direct observation to judge of the genotype of each male.

6. In the females the factors are always found cryptomerically, therefore all females look alike. In this respect inheritance in *Lebistes* is further sex-limited.

7. Crossing-over between the X- and the Y-chromosome in a male individual has been noted, by which colour factors are exchanged, and the sharp distinction between the X- and Y-chromosomes has thus been removed. The above described secondary male sex-characters in *Lebistes* are not a consequence of single factors, but of a series of linked factors.

Carlsberg Laboratory, Copenhagen, May 17, 1922.

Explanation of the Plates.

Plate 1 and plate 2 represent both *Lebistes reticulatus* and have been reproduced after a picture in oils.

Plate I.

4 uppermost rows: ♀ 333 and ♂ 334 with 9 ♂ youngs,
X_oX_o X_sY_m All X_oY_m

4 lower rows: ♀ 335 and ♂ 336 with 9 ♂ youngs,
X_oX_s X_oY_i $5X_sY_i + 4X_oY_i$

Plate II.

1. row: 3 ♂♂ of "Magdeburg Race" $= X_sY_r$
2. row: 3 F_1 males of ♀ 140 × ♂ 114 $= X_oY_r$
X_oX_o X_sY_r

3.—5. row: ♀ 337 and ♂ 338 with 6 ♂ youngs,
X_oX_s X_oY_m $3X_oY_m + 3X_sY_m$

The remaining figures at the bottom of Plate 2 represent schematically the effects produced by the various factors in the X and Y-chromosomes, both singly and in combination.

row 6:	Y_r	X_s	X_sY_r
row 7:	Y_i	Y_m	Y_f
row 8:	X_sY_i	X_sY_m	X_o

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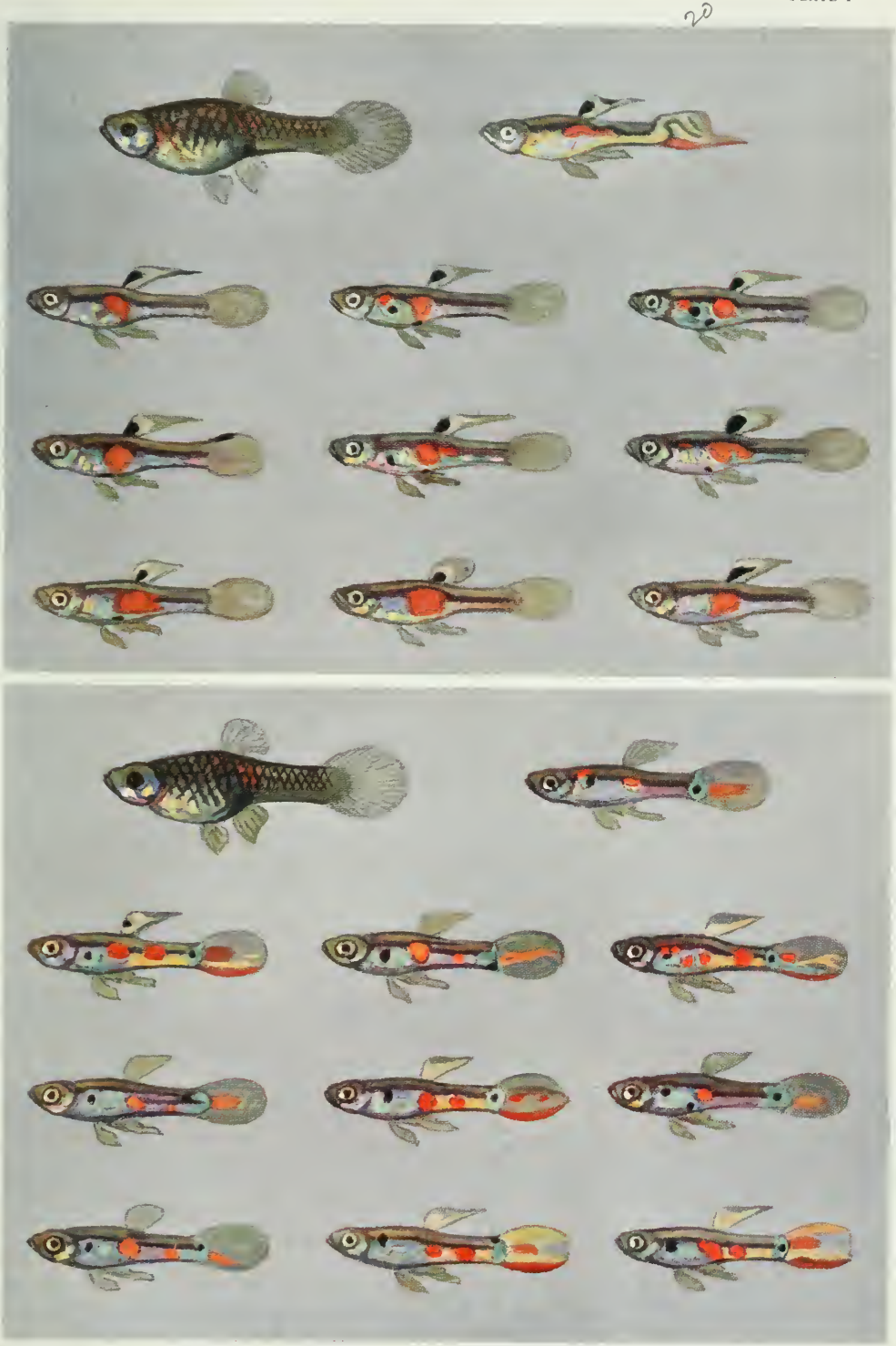

Ö. WINGE: Inheritance in *Lebistes reticulatus*.

O. WINGE: Inheritance in *Lebistes reticulatus*.

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JOHS. SCHMIDT:

RACIAL INVESTIGATIONS.

IX. CONTINUED STATISTICAL INVESTIGATIONS WITH *ZOARCES VIVIPARUS* L.

BY

KIRSTINE SMITH.

I. Introduction.

In a previous memoir[1]) the results of investigations with regard to inheritance in *Zoarces* from the station Nakkehage in Ise-Fjord were examined. Similar material from the station Langholm in Roskilde Fjord (in the northern part of the Danish island Sealand) has now been handed to me for statistical treatment by Dr. Johs. Schmidt. The characters investigated are: number of vertebrae (»Vert«), number of rays in the right pectoral fin (»Pd«), and number of pigment spots (Pigm. D_1), and they have been described by Dr. Schmidt[2]). The material consists of six samples taken late in the autumn of each of the years 1915—1920, when the females contain well developed embryos.

In addition I shall supply in a separate chapter a more detailed mathematical treatment of some results already given by Dr. Johs. Schmidt concerning inheritance under transplantation[3]). A sample of *Zoarces* from Nakkehage was put into boxes placed in the neighbourhood of the station, but under somewhat different conditions of temperature and salinity of the water. The transplantation was made at a season, when the sexual products of *Zoarces* were still quite immature, so that the embryos were subjected to the new environmental conditions from the earliest stage.

[1]) K. Smith: Comptes Rendus des Trav. du Lab. Carlsb. **14**, No. 11, 1921.

[2]) Johs. Schmidt: Compt. Rend. des Trav. du Lab. de Carlsb. **13**, No. 3 1917, p. 287.

[3]) Johs. Schmidt: Compt. Rend. des Trav. du Lab. Carlsb. **14**, No. 9, 1920 p. 6.

All the samples were collected by Capt. G. Hansen, who, together with the attendant of the Laboratory Mr. N. R. Poulsen, has also been responsible for the work of assigning serial numbers, skeletonising, and staining of the fishes. The countings have been made by Dr. Johs. Schmidt, mag. scient Vilh. Ege, and cand. mag. A. Strubberg. I have heartily to thank Miss A. G. Davin, Ireland, for linguistic revisal of my english manuscript.

II. Material and Methods.

The present material from Langholm for the investigation of inheritance differs from the material from Nakkehoved in that the age of the mothers has been determined and also in the fact that for the characters Vert and Pd only 5 embryos from each mother have been considered. By examining all the individuals of the Langholm samples, both males, females, and embryos, Dr. Johs. Schmidt has proved that the mean values of the racial characters[1]), at any rate for Vert and Pd, vary somewhat from one annual group to another. The present material has therefore been sorted according to the age of mothers as well as to the annual group of embryos, so that the same group only contains mothers of one annual group with embryos developed in one and the same year. It thus becomes possible to elucidate to what extent the calculated maternal correlation coefficient is influenced by possibly non-inherited somatic differences which may characterize individuals developed in different years and therefore in somewhat different environments. For each of the groups of observations so obtained a maternal correlation table was formed, but these tables will not be given here, because the results show, that it is permissible to group all the material together in calculating the maternal correlation coefficient.

In the memoir[2]) dealing with the Nakkehage material it was proved, that the characters considered had the same mean values and standard deviations for both sexes except for Pigment, where there was a small and doubtful deviation between the St. D.'s for males and females. The lack of knowledge concerning the sex of the embryos is therefore immaterial. In the same memoir the distribution of frequencies for mothers is illustrated

[1]) Johs. Schmidt: Compt. Rend. des Trav. du Lab. Carlsb. **14**, No. 15, 1921, p. 5.
[2]) l. c. pp. 2—4.

by examples and found to be described by the normal law of frequencies.

Also, with regard to the formulae used we can in most cases refer to the previous investigation, but the list of formulae now includes those for the probable errors of fraternal and maternal correlation coefficients and we will give the complete list of

Notations and Formulae:

Individual values for mothers x

Individual values for offspring ..... y

or for individuals of the p^{th} group of offspring $y_{p,1}, y_{p,2} \cdot y_{p,q}$

with mean value ..... y_p

Number of mothers ..... n

Number of embryos ..... nq

Mean value for mothers ..... $a_1 = \frac{\Sigma x}{n}$

Mean value for embryos ..... $a_2 = \frac{\Sigma y}{nq}$

Standard deviation for mothers from a_1 ... $\sigma_1 = \sqrt{\frac{\Sigma (x-a_1)^2}{n}}$

— — · — · b_1 $_b\sigma_1 = \sqrt{\sigma_1^2 + (a_1 - b_1)^2}$

— — · embryos from a_2 ... $\sigma_2 = \sqrt{\frac{\Sigma (y-a_2)^2}{nq}}$

— — · — · b_2 $_b\sigma_2 = \sqrt{\sigma_2^2 + (a_2 - b_2)^2}$

Product mom. of sec. order about a_1 and a_2. $\mu_{11} = \frac{1}{nq} \Sigma (x-a_1)(y-a_2)$

— — · · · — b_1 and b_2. $_b\mu_{11} = \mu_{11} + (a_1 - b_1)(a_2 - b_2)$

St. D. of means for groups of offspring .... $\sigma_q = \sqrt{\frac{\Sigma (y_p - a_2)^2}{n}}$

Maternal correlation coefficient ..... $r_m = \frac{\mu_{11}}{\sigma_1 \sigma_2}$

Fraternal correlation coefficient .... $r_f = \frac{2S\Sigma (y_{p,1} - a_2)(y_{p,2} - a_2)}{nq(q-1)\sigma_2^2}$

$= \frac{1}{q-1} \left(\frac{q \sigma_q^2}{\sigma_2^2} - 1 \right)$ [1])

Regression coefficient on mothers ..... $\alpha_m = \frac{\mu_{11}}{\sigma_1^2}$

---

[1]) Vide K. Smith: l. c. p. 8, where the formula is deduced for $q = 10$.

Regression coefficient on embryos ..... $a_e = \frac{\mu_{11}}{\sigma_2^2}$

Probable error of r_m

$$\left\{P.e.(r_m)\right\}^2 = 0.67449^2 \left\{\frac{(1-r_m^2)^2}{n} - \frac{q-1}{nq}(1-r_f)\left[1-r_m^2\frac{3-r_f}{2}\right]\right\}^{1)}$$

Probable error of r_f

$$P.e.(r_f) = 0{,}67449\sqrt{\frac{2}{nq(q-1)}}(1-r_f)(1+(q-1)r_f)^{1)}$$

Probable error of a_m

$$\left\{P.e.(a_m)\right\}^2 = 0.67449^2 \frac{\sigma_2^2}{nq\,\sigma_1^2}\left\{1+(q-1)\,r_f - qr_m^2\right\}^{1)}$$

III. Investigations of Inheritance in the Langholm population.

A. Maternal Correlation.

Vertebrae.

When the material is sorted in such a way that the same group contains only mothers of a single annual group belonging to one and the same sample, we get, by neglecting groups which have very few individuals, 16 groups of observations of mothers with 5 embryos. For each of these groups, means and moments of the second order have been calculated and the results are given in Table 1.

A comparison of the means for mothers or for embryos belonging to the same annual group shows that such means are approximately equal, while different annual groups are characterized by greater or smaller average values. This is demonstrated still more clearly in Dr. Johs. Schmidt's[2]) table, which also includes males and non-gravid females. Table 2 gives a summary of this table and also the means for embryos and fully developed individuals. It shows that the means for embryos are in every case somewhat greater that those for fully developed individuals, but that the deviation is only in one case greater than twice the probable error of the deviation. Comparing the

[1]) K. Smith: Biometrika **14**, 1922, pp. 17 and 9.
[2]) l. c. p. 5.

Table I. Vertebrae. Means and Moments of second order.

Annual group of mothers		1912	1913	1914	1915	1916	1917	1918	1919
Annual group of embryos	a_1	108.2500	107.8780	107.8974					
	σ_1^2	3.8875	3.0827	2.3997					
1916	a_2	108.5000	108.1951	108.0051	—	—	—	—	—
	σ_2^2	3.8500	3.5717	3.4513					
	μ_{11}	2.2350	1.8140	0.4723					
	n	20	41	39					
	a_1			107.8000	107.9241				
	σ_1^2			4.6933	5.0575				
1917	a_2	—	—	107.6933	107.6557	—	—	—	—
	σ_2^2			3.8126	3.9777				
	μ_{11}			3.0720	1.9637				
	n			15	79				
	a_1				108.1594	108.1200			
	σ_1^2				4.0181	3.0656			
1918	a_2	—	—	—	107.7362	107.6400	—	—	—
	σ_2^2				4.1014	4.9024			
	μ_{11}				1.6739	1.4832			
	n				69	25			
	a_1				108.0000	108.1341			
	σ_1^2				4.0000	3.5308			
1919	a_2	—	—	—	108.3463	108.2171	—	—	—
	σ_2^2				3.5532	3.0236			
	μ_{11}				1.4585	1.2416			
	n				41	82			
	a_1					107.8750	107.7750	107.2105	
	σ_1^2					3.2344	4.1244	2.4820	
1920	a_2	—	—	—	—	108.5500	108.0150	107.6737	—
	σ_2^2					3.7350	3.6548	4.4935	
	μ_{11}					1.1563	1.6683	1.1213	
	n					32	40	19	
	a_1					107.1000	107.8077	107.6753	108.0625
	σ_1^2					1.4900	4.0784	3.2582	4.4336
1921	a_2	—	—	—	—	107.4600	108.4077	107.8078	107.9750
	σ_2^2					4.6084	4.1030	3.5786	3.7494
	μ_{11}					0.2340	1.8169	1.1246	2.1141
	n					10	26	77	16

Table 2. Langholm. Vertebrae. Means for Annual Groups with probable error.

Annual group	Develop. Individuals	Embryos	All Individuals
1912	107.837±0.181	—	—
1913	107.847±0.157	—	—
1914	108.024±0.102	—	—
1915	108.138±0.041	—	—
1916	108.161±0.042	108.270±0.067	108.184±0.037
1917	107.670±0.069	107 783±0.093	107.720±0.062
1918	107.667±0.055	107.723±0.084	107.682±0.047
1919	108.162±0.046	108.312±0.073	108.205±0.039
1920	107.779±0.066	108.101±0.096	107.883±0.054
1921	—	107.903±0.081	—

total means for annual groups, we find for example that the 1917-, 1918- and 1920-groups have essentially smaller means than the 1915-, 1916- and 1919 groups. Differences are found as great as from 4 to 9 times the p. e. of the differences.

The number of observed pairs in most of the groups of Table 1 is so small, that taken separately they cannot give satisfactory determinations of the correlation coefficient, and the question arrises, how the best determination of the correlation coefficient of the total material can be obtained. It depends partly upon the nature of the annual variation.

It appears from Table 1 that the variation of the means for the annual groups cannot have a purely genetic origin. When considering, for instance, the large means for embryos of the 1919-group it is clear at once that they cannot be explained by means of the average values of the mothers, which only exceed the general average by a much smaller amount. The annual variations must either directly or indirectly by selection be caused by the environmental conditions to which the mothers or embryos have been subjected. But two possibilities may be imagined (1), that these annual deviations from the total mean are inherited by the following generation, or (2), that this does not take place.

In the latter case the calculation of correlation for the total material ought to be carried out so that each of the moments of second order are calculated about the means of the annual

group in question both for offspring and for mothers. From these moments we calculate the moments for the total material by weighting the moments of each group by the number of pairs of observations contained in it.

Denoting the means of the annual groups to which the mothers and the embryos belong by a_m and a_e, we thus find for the total material the moments

$$\left.\begin{aligned} \Sigma_1^2 &= \frac{1}{S\,(n)}\,S\left\{n(\sigma_1^2 + (a_1-a_m)^2\right\} = \frac{Sn\,\sigma_1^2}{Sn} + \frac{Sn\,(a_1-a_m)^2}{Sn} \\ \Sigma_2^2 &= \frac{1}{S\,(n)}\,S\left\{n(\sigma_2^2 + (a_2-a_e)^2\right\} = \frac{Sn\,\sigma_2^2}{Sn} + \frac{Sn\,(a_2-a_e)^2}{Sn} \\ \text{og } M_{11} &= \frac{1}{S\,(n)}\,S\left\{n(\mu_{11} + (a_1-a_m)\,(a_2-a_e)\right\} = \frac{Sn\,\mu_{11}}{Sn} + \frac{Sn\,(a_1-a_m)\,(a_2-a_e)}{Sn}, \end{aligned}\right\} \quad (1)$$

where S indicates summation over all groups of observations. Accordingly we find for the maternal correlation coefficient

$$r_m = \frac{M_{11}}{\Sigma_1 \Sigma_2} \qquad (2)$$

If on the contrary the variations of annual groups of mothers are inherited, a_m in these formulae has to by replaced by the total mean for mothers.

When carrying out the calculation according to the last form given in the formulae (1), so that the two sums appear in the calculation, occasion is given to examine, whether the means of mothers and of offspring for the same group are correlated. Denoting the last sums occurring in Σ_1^2, Σ_2^2 and M_{11} by s_1^2, s_2^2 and m_{11} respectively, we find that

$$r'_m = \frac{m_{11}}{s_1 s_2} \qquad (3)$$

is the correlation coefficient between means of mothers and of offspring from the same group weighted according to the size of the group. As the correlation coefficient for individuals r'_m may be calculated under the two assumptions stated above.

For a homogeneous material the correlation coefficient between means of the two variables equals the correlation coefficient between individual values of the variables. To make our material

homogeneous we must however consider it as composed of groups of siblings with their mother. Between the mean for 5 siblings and their mother's value the correlation coefficient is

$$R_m = r_m \frac{\sigma_2}{\sigma_q}, \qquad (4),$$

where σ_q according to the formula for fraternal correlation[1]) is

$$\sigma_q^2 = \frac{\sigma_2^2}{5}(1 + 4r_f) \qquad (5)$$

From this is found

$$R_m = r_m \sqrt{\frac{5}{1 + 4r_f}},$$

which is therefore the value we should expect to find for r'_m, if means of groups in Table 1 do not show other irregularities than such which can be explained by the individually observed maternal correlation and eventually by a variation of the means of annual groups.

Besides the values r_m, r'_m, and R_m mentioned above we give in Table 3, the values of correlation coefficients r_m and r'_m calculated from moments about the total means for mothers and for embryos, as well as r_m calculated from moments about the means of the individual groups.

Table 3. Maternal Correlation. — Vertebrae.

Moments calculated for mothers about	Mean of group	Mean of annual group	Total mean	Total mean
for offspring about	Mean of group	Mean of annual group	Mean of annual group	Total mean
r_m	**0.4033**	**0.4028**[2])	**0.4016**	**0.4009±0.0160**
r'_m	—	0.59[2])	0.28	0.29
R_m	—	0.533[2])	0.531	0.530

[1]) Vide p. 3.

[2]) The sample: Mothers 1916, offspring 1919 is not used for this calculation, because the mean for mothers differs abnormally from the other individuals of the same annual group.

It will be seen that whatever point of view is adopted when calculating r_m, we have arrived at values the differences of which are very small compared with the p. e. calculated for the last

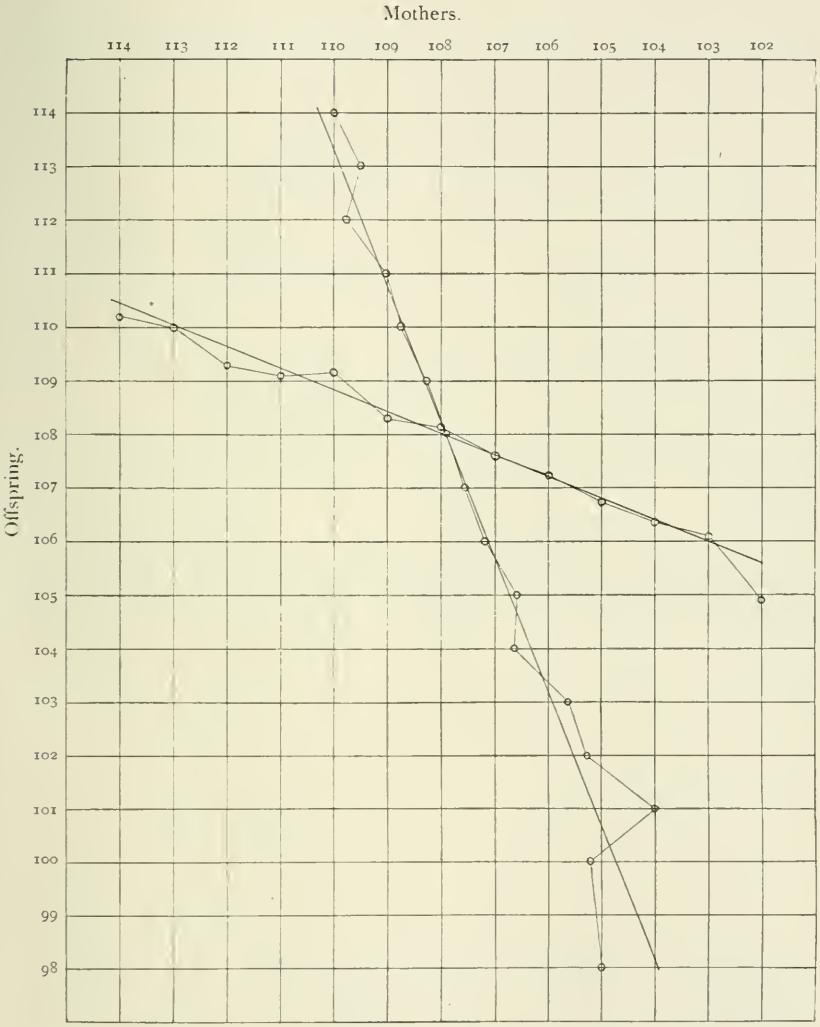

Fig. 1. Maternal Correlation. Vertebrae. Langholm 1915—1920. Regression on Mothers and on Offspring.

value given in Table 3. For this reason we shall, in the following, consider only that value which has the advantage of being directly calculable from a table of the total material (Table 17) and of having an easily calculated probable error.

The values of r'_m, which approximately coincide with the expected value R_m, show that the means for groups have decidedly genetic features, and that is true what ever assumption about the variation of annual means we may maintain. That is to say, the groups of Table 1 are too small to give separately a representative value of the mean of the annual groups to which its mothers or embryos belong.

For the total material (Tabel 17) the law of distribution is characterized by the values given in Table 11.

We find the two following regression lines:

Regression on mothers: $y - 107.9892 = 0.405688\ (x - 107.9144)$
— - offspring: $x - 107.9144 = 0.396224\ (y - 107.9892)$,

which are represented in Fig. 1.

For the ratios of correlation[1]) we find

for mothers $\eta_1^2 = 0.16682$ and for offspring $\eta_2^2 = 0.16356$,
furthers for mothers $\zeta_1 = \eta_1^2 - r_m^2 = 0.00607$ — — $\zeta_2 = \eta_2^2 - r_m^2 = 0.00282$

Assuming that, when our 3155 pairs of observations for calculating r_m give the same accuracy of r_m as 1225 independent pairs, the same is the case in the calculation of ζ, we find by Blakeman's formula[2])

$$\sigma_\zeta^2 = 4\,\frac{(\eta^2 - r^2)}{N}\left\{(1-\eta^2)^2 - (1-r^2)^2 + 1\right\}$$

the following values:

$$\sigma_{\zeta_1}^2 = 0.00001964, \qquad \sigma_{\zeta_2}^2 = 0.00000916$$

and p.e.$\zeta_1 = 0.00299$ p.e.$\zeta_2 = 0.00204$.

We have thus found values for ζ which for regression on offspring is 1.4 times and for regression on mothers 2.0 times the probable error of ζ. This value of ζ may signify a small deviation from linear regression.

In examining the material from Nakkehoved[3]) I found that

[1]) Karl Pearson: On the general theory of shew correlation. — Drapers' Company. Research Memoirs, Biometric Series II. 1905, p. 10.
[2]) Biometrika **4.** 1905, p. 339.
[3]) l. c. pp. 10—11.

ζ_1, was equal to 0.0091 and that it exceeded its probable error to a still higher degree, being three times the probable error. Dr. G. Duncker[1]) objected that I did not emphasize this deviation as opposed to the pure linearity of the regression curve on offspring. This led me to a more detailed examination of the material, from which I found[2]) that by neglecting a sample which

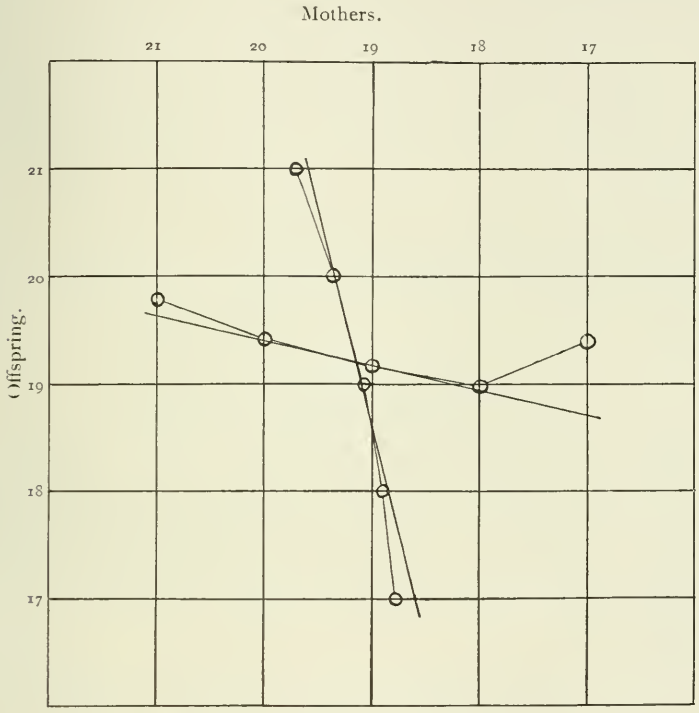

Fig. 2. Maternal Correlation. Pd. Langholm 1915—1920. Regression on Mothers and on Offspring.

deviated greatly from the rest the value of ζ_1 was reduced to twice its probable error.

It might be argued that the new result obtained, which also shows for the regression on mothers a certain deviation from linear form, might be a confirmation of the deviation being significant. By comparing Fig. 1 with Fig. 1[3]) of the previous paper it becomes evident, however, that the two figures show a decided want of agreement between the deviations of mean values from

[1]) l. c. p. 55.
[2]) l. c. p. 59.
[3]) l. c. p. 9.

regression lines. For large numbers of vertebrae the means in the two figures deviate decidedly in opposite directions, and neither does the tendency to a point of inflexion in the neighbourhood of the total means, which occurs in the Nakkehoved material, appear at all in the new material. The only resemblance to be found between the two figures is that the means corresponding to the smallest number of vertebrae deviate to the same side of the regression line. But this mean represents in the Langholm material only two mothers so that this agreement is not of great weight. The want of resemblance between the two figures indicates that the smaller deviations from linear form observed in regression curves on mothers do not signify and ought not to be considered typical.

I shall not therefore discuss further the form of the regression curve in this case but would only remind the reader that the calculation of the probable error of ζ does not rest on a sufficiently safe basis to justify too detailed investigations.

Rays in right pectoral fin (Pd.).

Table 4 gives means and moments of second order for Pd for groups of observations corresponding to those of Table 1. It is evident from this table, but still more so from Table 5, which contains a summary of Dr. Johs. Schmidt's determinations[1]) of means for annual groups, that this character shows even more marked deviations than does Vert between means for various annual groups.

It appears from Table 5 that the means for embryos and fully developed individuals of the same annual group agree quite well, except for the 1917-groups in which some selection appears to have taken place. For this annual group therefore in the following calculation we shall use the mean as found from embryos alone. In Table 5 very large differences are observed between means of annual groups as for example 0.43 ± 0.02. (between 1915- and 1916 groups).

From Table 4 we find that the mean of embryos belonging to the annual group 1916 is 0.47 greater than that for embryos of the 1917-group, although the difference between the means of mothers is only 0.11, from which it is clearly seen that the matter is not explained by inheritance.

[1] Compt.-Rend. du Lab. de Carlsb. **14**, No. 15, 1921, p. 7.

Table 4. Fd. Means and Moments of second order.

Annual group of mothers		1912	1913	1914	1915	1916	1917	1918	1919
Annual group of offspring	a_1	19.3000	19.0244	19.1250					
	σ_1^2	0.3100	0.4628	0.5094					
1916	a_2	19.4800	19.4585	19.1800	—	—	—	—	—
	σ_2^2	0.3096	0.2873	0.3076					
	μ_{11}	0.06600	0.09126	0.07250					
	n	20	41	40					
	a_1			19.3750	18.9367				
	σ_1^2			0.3594	0.3631				
1917	a_2	—	—	18.9250	18.8709	—	—	—	—
	σ_2^2			0.4194	0.4314				
	μ_{11}			0.15313	0.06525				
	n			16	79				
	a_1				19.0290	19.5200			
	σ_1^2				0.4339	0.3296			
1918	a_2	—	—	—	18.9420	19.0480	—	—	—
	σ_2^2				0.4198	0.3977			
	μ_{11}				0.10313	0.10304			
	n				69	25			
	a_1				18.8913	19.4643			
	σ_1^2				0.4012	0.3916			
1919	a_2	—	—	—	19.1652	19.1595	—	—	—
	σ_2^2				0.2771	0.3103			
	μ_{11}				0.03970	0.07355			
	n				46	84			
	a_1					19.4688	19.1500	18.8421	
	σ_1^2					0.4365	0.4275	0.3435	
1920	a_2	—	—	—	—	19.6813	19,6750	19.4421	—
	σ_2^2					0.3796	0.3094	0.3940	
	μ_{11}					0.14941	0.10375	0.12244	
	n					32	40	19	
	a_1					19.6000	19.3462	19.0779	19.0625
	σ_1^2					0.4400	0.3802	0.3316	0.3086
1921	a_2	—	—	—	—	19.3800	19.2692	19.2442	19.1750
	σ_2^2					0.2756	0.2583	0.3871	0.2694
	μ_{11}					0.13200	0.07604	0.10825	0.03906
	n					10	26	77	16

Table 5. Langholm. Pd. Means of Annual Groups with probable errors.

Annual group	Develop. Individ.	Embryos	All Individuals
1912	19.238±0.060	—	—
1913	19.067±0.050	—	—
1914	19.103±0.033	—	—
1915	18.997±0.012	—	—
1916	19.437±0.013	19.387±0.020	19.422±0.011
1917	19.214±0.020	18.910±0.032	—
1918	18.960±0.017	18.973±0.023	18.965±0.014
1919	19.168±0.015	19.154±0.019	19.163±0.012
1920	19.530±0.021	19.598±0.025	19.558±0.016
1921	—	19.239±0.022	—

Table 6. Maternal Correlation. — Pd.

Moments calculated for mothers about	Mean of group	Mean of annual group	Total mean	Total mean
for offspring about	Mean of group	Mean of annual group	Mean of annual group	Total mean
r_m	**0.2388**	**0.2408**	**0.2407**	**0.2406±0.0171**
r'_m	—	0.313	0.351	0.256
R_m	—	0.344	0.344	0.344

By using the same methods as for vertebrae the correlation coefficients given in Table 6 are found.

As in the case of Vert, the differences between the three values found for r_m, are considerably smaller than the probable error of the correlation coefficient calculated from the total material (Table 18), which is given in the last column of Table 6. The values of r'_m indicate a decided correlation between means of mothers and of offspring from the same group, which for the two first values of r'_m is approximately equal to the expected value R_m. This signifies that the means for groups do not exhibit irre-

gularities other than can be explained by inheritance and varying means for annual groups. The numerical results do not on the other hand give any help in deciding which of the two hypotheses used for calculating the two values of r_m is the right one.

For regression on mothers and on offspring we find from the total material (Table 18) the regression curves:

$$y - 19.20625 = 0.231993\ (x - 19.15938)$$
$$\text{and } x - 19.15938 = 0.249499\ (y - 19.20626),$$

which are represented in Fig. 2. (p. 11).

The squared ratios of correlation have the following values:

Regression on mothers

$$\eta_1^2 = 0.06137 \text{ and on offspring } \eta_2^2 = 0.05934.$$

For judging the linearity of the curves we find

$$\zeta_1 = \eta_1^2 - r_m^2 = 0.00348 \text{ and } \zeta_2 = \eta_2^2 - r_m^2 = 0.00146,$$

which, supposing that the 3200 pairs of observations in this case as in calculating r_m afford the same accuracy as 1368 independent pairs, have the probable errors

$$\text{p. e. } (\zeta_1) = 0.00214 \text{ and p. e. } (\zeta_2) = 0.00139.$$

Neither for this character do we find signs of significant deviations from linear regression.

We shall in the following endeavour to elucidate whether the variation of means for annual groups can be traced in the offspring. Has an annual group with a large average number of pectoral fin rays, offspring with a relatively large number of pectoral fin rays? Or, we are tempted to ask: Do the means for two groups of offspring from mothers belonging to two different annual groups with different mean values differ as much from each other as they would do if the mothers all belonged to the same annual group and had the same means as the present mothers have? This expected difference is, however, undefined until assumptions are made about the selection of the two groups of mothers. Has the selection taken place according to the somatic

values of the mothers only, so that for each given x no selection has taken place with regard to y, we must expect that the difference between the means of offspring is equal to the difference between the means of mothers multiplied by the regression coefficient on mothers not considering the possible difference between the two groups of fathers. A simpler basis of comparison is obtained by considering offspring from mothers with a given number of pectoral fin rays. In this way it is possible to examine whether two groups of offspring from the same year, which have mothers with the same somatic value but belonging to different annual groups have different means, or in other words if individuals with the same somatic value belonging to different annual groups have the same generative value on an average.

Among mothers the annual groups 1915 and 1916 are especially well represented, and as the means for these annual groups, as will be seen from Table 5, differ considerably, a comparison of the offspring from mothers of these two annual groups is well suited for such an examination. As no large groups of mothers of two annual groups occur in a single sample, we must, by correcting for differences between means of annual groups, refer all offspring to one year. We assume hereby that a given group of offspring, had it been developed in 1919 instead of in 1917, would have had a mean found by adding to the mean actually found the difference $(a_{19}—a_{17})$ between the means of the two annual groups in question.

In Table 7 are given simple as well as corrected means. When we consider for each number of pectoral fin rays in mothers the total mean values for the offspring, we notice that in all the three cases, the mean for offspring from the 1915-group is the greater. Weighting each of the three differences according to the number of observations we find that the average difference between offspring from mothers with the same somatic value is 0.043.

The probable errors have been directly calculated for offspring belonging to the 1919-group come from mothers with the value 19 for Pd.

For offspring from the 1915-group the mean is $19.100 \pm .043$,
and - — - - 1916-group - — - $19.057 \pm .041$,
so that the difference is 0.043 ± 0.060.

Table 7. Pd. Mean Values for Offspring from the annual groups 1915 and 1916 for given value of the mother. *(Numb. of embr. in italics).*

Mother . . .	21	20	19	18	17	21	20	19	18	17
Annual group of offspring	Plain means for offspring from the 1915-group					Means referred to 1919 for offspring from the 1915-group				
1917	»	19.1167	18.8560	18.7412	»	»	19.370 *60*	19.109 *250*	18.994 *85*	»
1918	»	19.2000	18.9128	18.7286	»	»	19.398 *80*	19.111 *195*	18.927 *70*	»
1919	»	19.5000	19.1000	19.1333	19.4000	»	19.500 *30*	19.100 *150*	19.133 *45*	19.400 *5*
1917—1919							**19.406** *170*	**19.107** *595*	**19.002** *200*	19.400 *5*
	Plain means for offspring from the 1916-group					Means referred to 1919 for offspring from the 1916-group				
1918	19.6000	19.1818	18.8923	»	»	19.798 *5*	19.380 *55*	19.090 *65*	»	»
1919	19.3333	19.2833	19.0571	18.9333	»	19.333 *15*	19.283 *180*	19.057 *210*	18.933 *15*	»
1920	20.8000	19.8133	19.5000	19.4000	»	20.405 *5*	19.418 *75*	19.105 *70*	19.005 *10*	»
1921	20.0000	19.4000	19.2400	»	»	19.924 *5*	19.324 *20*	19.164 *25*	»	»
1918—1921						19.688 *30*	**19.333** *330*	**19.079** *370*	**18.962** *25*	

Starting from this probable error and using various approximations we find that the probable error of the total average difference 0.043 between offspring from mothers with the same somatic value is 0.029. This probable error is so large that no significance would be given to the difference if it were not so that the individual values of Table 7 shew such regular deviations in the same direction. This applies not only to the total mean for given value of mother, but also when we consider the annual groups 1918 and 1919, which are directly comparable, and for which therefore the uncertainly arising from correction for annual groups is avoided, we notice that in each pair of values the mean for offspring from the 1915-group is the greater.

There are thus several indications pointing to the fact that if one compares individuals with the same somatic value belonging to two annual groups the means of which differ widely, it will be found that the individuals belonging to the annual group with the larger mean have on an average a smaller generative value. We have not considered a possible difference between the probable means for fathers, but it is probable that the mean for the males, which have mated the 1915-group consisting of females with large mean values, is larger than that for those which have mated the 1916-group with the smaller value. When the offspring from the 1915-group has nevertheless the greater mean value. it must be expected that if we were able to compare offspring of parents belonging to the 1915-group with offspring of parents from the 1916-group for given values of both parents, their mean values would differ still more than the means of Table 7.

We shall next look at the total means for the two groups of mothers and their offspring and return to the first question: Do the groups of offspring differ so much as would be expected when the mothers had the present somatic values, all belonged to the same annual group, and the two groups of mothers had been selected only according to values of mothers? If the regression curves of mothers for each of the two groups of observations be fairly linear, the answer is already given by the foregoing result.

The mean for mothers is for the 1915-group $18.959 \pm .034$
and for the 1916-group $19.483 \pm .031$
with the difference $-0.525 \pm .0461$

The mean for the other individuals known is for

the 1915-group 19.005±.013
and for the 1916-group 19.415±.011
with the difference — 0.410±.017.

The mean for offspring referred to 1919 is

for offspring from the 1915-group 19.139
and - — - - 1916-group 19.210.

These means are because of the corrections for annual groups correlated, an exact calculation gives for their difference 0.071 the probable error 0.028.

As the difference between the means for the two groups of mothers is 0.525, we must, with the regression coefficient 0.2320±.0169 expect the difference between means of offspring to be 0.122±(0.014)[1]). We thus find between the actual and expected difference the deviation

$$0.122 - 0.071 = 0.051 \pm (.031)$$

approximately agreeing with the average deduction of 0.043 which we arrived at for constant value of mother.

The figures above show that the two groups of mothers differ more from each other than do the other individuals known of their annual groups. It might be asked if the deduction found is sufficient to counterbalance the difference expected between the offspring of mothers the means of which coincide with those of the annual groups. When the difference between means of mothers is made equal to 0.410, we find that the difference expected for offspring is

$$0.095 \pm (.008),$$

so that $0.095 - 0.043 = 0.052 \pm (.029)$.

This result suggests therefore that the difference between annual groups shown by different mean values can be traced to some extent in the means of the offspring, but not so much as the same differences occurring in mothers belonging to the same annual group would be traceable.

The uncertainty of the results of this investigation is so great

[1]) When in the following a probable error is put into brackets this indicates that the value is given approximately. In this case, for instance, the correlation of the two factors is not taken into account.

that I should not have given them here if there were much prospect of acquiring in the near future a better material of *Zoarces* for such an investigation. To obtain certain results 8—10 times as many mothers would be required even from annual groups differing as much from each other as the present ones, and annual groups differing as much as these do not often favour the investigation.

For vertebrae the conditions for a similar investigation are even more unfavourable because the means of annual groups vary less in proportion to the St. D. within an annual group. On the other hand the regression coefficient is greater for Vert than for Pd., but is not large enough to outweigh the disadvantage mentioned.

Pigment D_1.

The counting of pigment spots has only been done for 4 of the samples mentioned in the foregoing. From each group of offspring 10 embryos have been examined. The means and moments of second order for the 9 available groups of pairs of observations are given in Table 8, while the means for annual groups

Table 8. Pigment D_1. Means and Moments of second order.

Annual group of mothers		1914	1915	1916	1917	1918
Annual group of offspring 1917	a_1	12.1250	12.7222			
	σ_1^2	1.8594	2.9043			
	a_2	12.1875	12.4204	—	—	—
	σ_2^2	3.1773	3.4363			
	μ_{11}	1.3141	1.3779			
	n	8	54			
1918	a_1		12.6667	12.0000		
	σ_1^2		3.1852	2.0000		
	a_2	—	12.3352	12.1500	—	—
	σ_2^2		3.3673	2.7942		
	μ_{11}		1.1673	0.5944		
	n		54	18		
1919	a_1		12.4545	12.3939		
	σ_1^2		3.0358	2.3600		
	a_2	—	12.4576	12.4985	—	—
	σ_2^2		2.7088	2.3955		
	μ_{11}		1.0587	.7461		
	n		33	66		
1920	a_1			12.1379	12.3793	12.0000
	σ_1^2			2.1879	4.0285	2.7692
	a_2	—	—	12.0655	12.0310	11.3077
	σ_2^2			2.3302	2.6990	2.7053
	μ_{11}			0.5599	1.2641	0.4923
	n			29	29	13

Table 9. Langholm. Pigment. D_1. Means for Annual Groups with probable errors.

Annual group	Develop. Individuals	Embryos	All Individuals
1914	12.175±0.167	—	—
1915	12.729±0.040	—	—
1916	12.442±0.044	—	—
1917	12.456±0.074	12.404±0.083	12.433±0.055
1918	12.412±0.059	12.397±0.067	12.405±0.044
1919	12.500±0.068	12.448±0.060	12.471±0.045
1920	—	11.911±0.069	—

according to Dr. Johs. Schmidt's material of all individuals examined are given in Table 9.

It will be seen from Table 9 that the means for the annual groups 1916—1919 practically agree, while 1915 is characterized by a particularly large and 1920 by a particularly small mean value. Other groups do not occur in this investigation. The difference between means for 1915 and 1920 is 0.818±0.080.

Proceeding as in the case of Vert and Pd. we find for the correlation coefficients the values given in Table 10.

Table 10. Maternal Correlation. — Pigment D_1.

Moments calculated for mothers about	Mean of group	Mean of annual group	Total mean	Total mean
for offspring about	Mean of group	Mean of annual group	Mean of annual group	Total mean
r_m	**0.3506**	**0.3514**	**0.3495**	**0.3560±0.0213**
r'_m	—	0.43	0.29	0.60
R_m	—	0.523	0.521	0.530

Also for this character the different values for r_m coincide so closely that we may use the value which is found from a table

of the total material (Table 19). From this table the two regression lines are found:

$$\text{on mothers } y - 12.28553 = .360903\ (x - 12.43421)$$
$$\text{and - offspring } x - 12.43421 = .351148\ (x - 12.28553),$$

which are represented in Fig. 3.

For judging the linearity we have for

$$\text{mothers } \zeta_1 = \eta_1^2 - r_m^2 = 0.01158$$
$$\text{and for offspring } \zeta_2 = \eta_2^2 - r_m^2 = 0.00492,$$

and counting the 3040 pairs of observations as equivalent to 762 independent pairs in the calculation of ζ as well as that of r_m we find

$$\text{p. e. } (\zeta_1) = 0.00526 \text{ og p. e. } (\zeta_2) = 0.00341.$$

We therefore find in this case also only smaller deviations from linear form.

It will be seen from the Tables 3, 6, and 10 that whichever of the four methods of calculation we use for determining the correlation coefficient r_m, we arrive at almost the same result. Comparing the first three results with the correlation coefficient obtained directly from tables of the total material we find differences which for the three characters amount at the most to 0.18, 0.12, and 0.31, times respectively the p. e. of this r_m. We can therefore content ourselves with this value, for which we know the probable error.

Table 11 gives the constants obtained from the total correlation tables. These results will, together with those for the fraternal correlation coefficient, be discussed and compared with the results from the Nakkehoved material.

Table 11. Langholm. Variation and Correlation between mothers and offspring.

	qn	$a_1 \pm$ s.F.(a_1)	$a_2 \pm$ s.F.(a_2)	σ_1	σ_2	$r_m \pm$ s.F.(r_m)	r_m with Sheppard's correc
Vert.	5×631	107.914±0.052	107.989±0.040	1.93695	1.95995	0.4009±0.0162	0.4099
Pd.	5×640	19.159±0.018	19.206±0.012	0.66349	0.63979	0.2406±0.0172	0.2994
Pigm. D_1	10×304	19.434±0.065	12.286±0.045	1.69041	1.71374	0.3560±0.0213	0.3665

B. Fraternal Correlation.

The means and standard deviations for embryos are already given in Table 2, 5, and 9, and we can limit the investigation

Fig. 3. Maternal Correlation. Pigment D_1. Langholm 1916—1919. Regression on Mothers and on Offspring.

here to the fraternal correlation coefficients and the form of the regression line.

For the calculation of the fraternal correlation coefficient all embryos from the same sample can be taken together. The calculation is based upon the formula

$$r_f = \frac{1}{q-1}\left(q\,\frac{\sigma_q^{\,2}}{\sigma_2^{\,2}} - 1\right),$$

which gives an identical result with that obtained from a symmetrical fraternal correlation table in which each pair of brothers is entered twice.

In Tables 20—22 we give the distribution of frequencies for individual values of embryos with corresponding $\sigma_2{}^2$ and in Tables 23—25 the distribution of frequencies for means of groups of siblings together with σ_q^2. The fraternal correlation coefficients with probable errors calculated from the formula

$$\text{p. e. } (r_f) = 0.67449 \sqrt{\frac{2}{nq\ (q-1)}\ (1-r_f)\ (1 + r_f\,(q-1))}\ ^{1})$$

are given in Table 12.

Table 12. Fraternal Correlation.

Character	Vert.	Pd.	Pigm. D_1
Sample taken in:			
1915	0.4925±0.0199	0.4123±0.0198	—
1916	0.4541±0.0304	0.4850±0.0297	0.4542±0.0257
1917	0.4423±0.0273	0.3344±0.0274	0.4155±0.0233
1918	0.4210±0.0272	0.2406±0.0260	0.2911±0.0240
1919	0.5085±0.0287	0.3102±0.0297	0.4070±0.0252
1920	0.4415±0.0269	0.3601±0.0272	—
All observ.[2])	0.4637±0.0106	0.3609±0.0106	0.3898±0.0128

Fraternal correlation coefficients for the various samples do not, on the whole, differ more from each other than would be expected from the values of the probable errors. The only exception to this is the sample from 1918 containing embryos of the annual group 1919 for which the correlation for Pd., as well as for Pigment is unusually small. I have not been able to find any reason for this.

In order to investigate the form of the regression curves I have, for a single sample, formed the fraternal correlation table for each of the characters. (Tables 26—28). The values for $\zeta = \eta^2 - r_f^2$ are given in Table 13. By calculating σ_ζ a similar

[1]) Kirstine Smith: Biometrika **14.** 1922.

[2]) With Sheppard's correction for discontinuity r_f takes the values: Vert. 0.4743, Pd. 0.4696, and Pigm. D_1 0.4015.

proceeding is followed as for the maternal regression lines, that is, in Blakeman's formula (vide p. 10) we have for the number of observed pairs of observation introduced the number of inde-

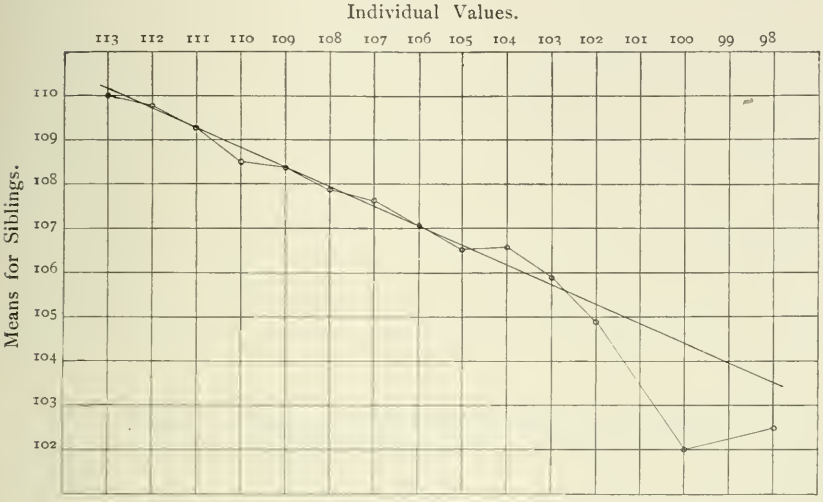

Fig. 4. Fraternal Regression Line. Vertebrae. Langholm. Annual group: 1921.

pendent pairs which, in calculating r_f would give the same accuracy as the present observations.

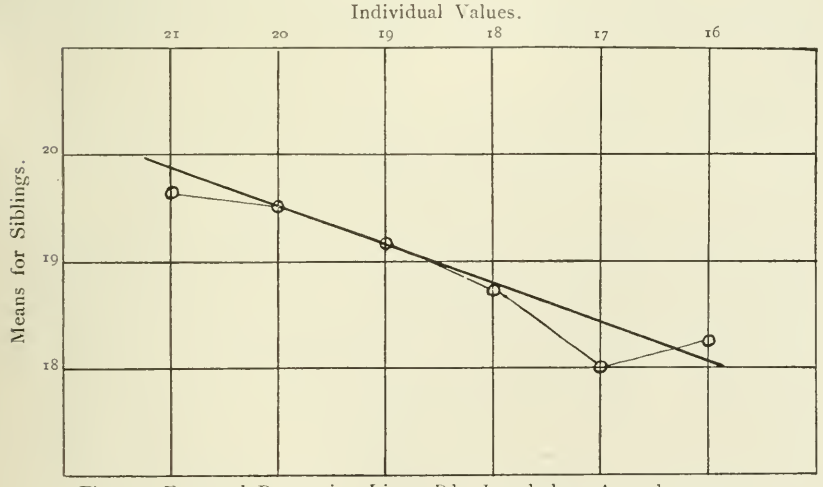

Fig. 5. Fraternal Regression Line. Pd. Langholm. Annual group: 1921.

It will be seen from the table that only for Vert does ζ exceed its probable error, and even in this case it is only 1.3 times the probable error.

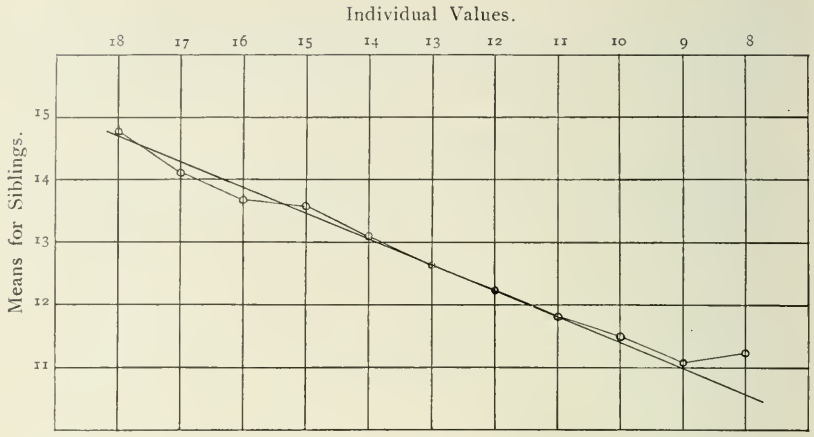

Fig. 6. Fraternal Regression Line. Pigment D_1. Langholm. Annual group: 1918.

Table 13. Linearity of Fraternal Regression Curves.

Character	Sample taken in	Numb. of obs. pairs	Numb. of independ. pairs equivalent to observ. pairs	$\zeta \pm$ p.e(ζ)
Vert.	1920	10×150	407	0.00706±0.00558
Pd.	1920	10×150	466	0.00366±0.00377
Pigm. D_1	1917	45×143	574	0.00224±0.00266

C. Comparison with the Investigation of Inheritance in the Nakkehoved Population.

It appears from a comparison of the means in Table 11 and the corresponding values for Nakkehoved[1]) that the number of Vert for the Langholm population is, on an average, more than 5 units smaller, and that the average number of pigment spots at this place is about $1^1/_2$ less than for Nakkehoved. Between the means for Pd there is no essential difference; the standard deviation for the two populations are also about equal for Vert and for Pd, while for Pigment there is a small difference. The maternal correlation coefficients for the two populations are compared in Table 14.

[1] Kirstine Smith: l. c. pp. 17—20.

Table 14. Maternal Correlation. Nakkehoved and Langholm.

Character	Vert.	Pd.	Pigm. D_1
Nakkehoved 1914—1919[1])	0.4021±0.0131[3])	0.2654±0.0133[3])	0.3654±0.0158[3])
Langholm 1915—1920[2])	0.4009±0.0162	0.2406±0.0172	0.3560±0.0213
Diff. (Nakkehoved÷Langholm)	+0.0012±0.0208	+0.0248±0.0217	+0.0094±0.0265
½ (Nakkehoved+Langholm)	**0.4015**±0.0104	**0.2530**±0.0109	**0.3607**±0.0133

It will be seen that the maternal correlation coefficients agree closely for Vert and Pigm., and for Pd they differ only by 1.1 times the probable error of the difference.

Table 15. Fraternal Correlation. Nakkehoved and Langholm.

Character	Vert.	Pd.	Pigm. D_1
Nakkehoved 1914—1919[1])	0.4689±0.0095[3])	0.3564±0.0092[3])	0.3517±0.0122[3])
Langholm 1915—1920[2])	0.4637±0.0106	0.3609±0.0106	0.3898±0.0122
Diff. (Nakkehoved÷Langholm)	+0.0052±0.0142	÷0.0045±0.0140	÷0.0381±0.0173
½ (Nakkehoved+Langholm)	**0.4663**±0.0071	**0.3587**±0.0070	**0.3708**±0.0086

Table 15 shows that the fraternal correlation coefficients for Vert and Pd. in the two populations also agree, and for Pigment a difference is found of 2.2 times its probable error. If however we calculate directly from the four annual values for Langholm, the probable error of their mean, we arrive, owing to the deviating value for 1918 at the greater value 0.031, and if this be used in the calculation, the probable error of the difference between the fraternal correlation coefficient for Langholm and for Nakkehoved is found to be 0.033, which is about as large as the difference itself.

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[1]) For Pigment 1915—1919.

[2]) For Pigment 1916—1919.

[3]) See Biometrika **14** 1922 pp. 12 og 21, where the calculation is carried out by the formula p. 4, which was not available when the value was first calculated.

We have thus found for all the three characters from the Langholm material, maternal and fraternal correlation coefficients which agree closely with the results obtained from the Nakkehoved material, although the mean values for Vert. and Pigment differ very much in the two populations. It has therefore been confirmed that both of the correlation coefficients for *Zoarces* are decidedly smaller than those which are usually found for physical characters in man, horse and dogs. A detailed discussion of the numerical values was given in the former memoir pp. 24—25 and 27—28.

IV. Investigation of Inheritance with Transplanted Individuals.

Dr. Johs. Schmidt has already communicated[1]) the chief results of the experiment of transplantation, but a more detailed numerical treatment of them will be given here. The conditions of experiment need only be stated briefly. In the summer 1917, at a season when the sexual products of *Zoarces* are not yet mature, about 600 individuals of this fish from Nakkehage (Station 31) were put into two big wooden boxes which were placed in an enclosed area in the neighbourhood of this station. The mating and the development of the embryos then took place in captivity. The physical conditions in the boxes and at the station differed, there being a higher temperature and a smaller salinity in the boxes than at the station 31.

Of the approximately 600 individuals captured abt. four fifth were females. At the end of the experiment (December 1917) the boxes contained 93 gravid females 47 females without embryos and 47 males. As far as possible 10 embryos from each mother were examined. The results are given in the Tables 29—33.

The usual calculation of correlation gives the results stated in Table 16, which for purposes of comparison also contains the constants deduced from a sample taken at Station Nakkehage[2]) in the same autumn.

[1]) Compt. Rend. des Trav. du Lab. de Carlsb. **14**, No. 6, 1920 pp. 6—7 and No. 15, 1921, pp. 2—5.

[2]) K. Smith: l. c. pp. 17—20 and p. 26.

Table 16. Nakkehage. 1917 — Sample and Transplantation Experiment.

	Numb. of moth.	Mean for moth. ± p. e.	Mean for embr. ± p. e.	St. D. for moth. ± p. e.	St. D. for embr. ± p. e.	Matern. Corr ± p. e.	Fratern. Corr. ± p. e.
Free	177	113.102±0.093	113.248±0.075	1.838±0.066	2.035±0.040	0.3775±0.0295	0.4715±0.0209
Vert. Capt.	88	112.648±0.140	114.450±0.116	1.937±0.099	2.140±0.064	0.3535±0.0677	0.5237±0.0285
Free	176	19.250±0.031	19.102±0.019	0.608±0.022	0.583±0.010	0.2824±0.0294	0.3634±0.0206
Pd. Capt.	92	19.174±0.040	19.757±0.047	0.564±0.028	0.856±0.026	0.2479±0.0501	0.5519±0.0280
Free	127	13.677±0.097	13.502±0.067	1.616±0.068	1.625±0.035	0.3722±0.0332	0.4112±0.0247
Pigm.D[1] Capt.	77	13.610±0.121	13.117±0.081	1.564±0.085	1.607±0.041	0.2983±0.0440	0.3681±0.0312

The deviation between means for embryos from free and those from captured mothers has been discussed by Dr. Johs. Schmidt in the two memoirs mentioned above. It appears clearly from Table 16 that in embryos the mean value has increased for Vert and decreased for Pd. and Pigment as a result of the altered environment. The standard deviations in the offspring are for Vert and Pigm. essentially the same for free and captured individuals, but for Pd the standard deviation has increased very much in the offspring of captured individuals. This is also the case with the fraternal correlation coefficient for Pd, while for Vert and Pigm. it is not affected by the altered environment.

There exists between r_f and σ_2 the relation

$$r_f = \frac{1}{9}\left(\frac{10\,\sigma_q^2}{{\sigma_2}^2} - 1\right) = 1 - \frac{{}_p\sigma_f^2}{{\sigma_2}^2}1),$$

where ${}_p\sigma_f$ is the presumptiv standard deviation for a fraternity. It follows from this that for Pd σ_q^2 must have increased proportionately more than ${\sigma_2}^2$. We find in fact for σ_q and ${}_p\sigma_f$ the following values for offspring of free and captured individuals:

	σ_q	${}_p\sigma_f$
Free	0.3808	0.4649
Captured	0.6609	0.5727

[1]) l. c. p. 29.

It will be seen from this that the standard deviation inside a fraternity has only increased feebly compared with what the standard deviation for means for decades has increased. In other words, offspring of one mother have reacted in a more similar way towards the altered environment than have the different fraternities.

Between the maternal correlation coefficients for free and for captured individuals, no significant deviations are found. It is true that r_m for all the three characters is smaller for captured than for free individuals, but for Vert and Pd the deviations are smaller than their probable errors, and for Pigm. it is only 1.3 time the probable error.

For Pd we find, on account of the large St. D. for embryos of captured individuals, a large regression coefficient on mothers viz. 0.376, but it has a probable error of 0.078 so that the difference between it and the value 0.271 ± 0.029 found under natural conditions is only 1.3 times the probable error of that difference. The regression curve is decidedly linear.

While the distribution of frequencies for offspring of captured *Zoarces* for Vert and Pigm. is practically symmetrical, the distribution for Pd is decidedly skew. Calculated for 1000 individuals we find for embryos from Nakkehoved 1914—1919 and from captured *Zoarces* 1917 the following distribution:

Number of Pd.	21	20	19	18	17	16	15
Free 1914—1919	11	299	608	82	1	0	0
Captured 1917	3	149	543	226	66	8	4

It appears from this table that the decrease in the mean is not only due to occurrence of individuals with 15 or 16 rays, which have not been observed elsewhere but also to a general displacement of frequencies of 21, 20, and 19 rays to the lower numbers.

As the means for Vert and Pd under the new environment have altered so greatly in opposite directions the question arises whether an individual negative correlation can be traced between the values of Vert and Pd in the offspring of transplanted individuals. From the correlation table (Table 34) it appears that

the value found, —0.031, is so small that no significance can be attached to it. Even if the calculation had been undertaken by means of unrelated individuals its St. D. would have been as great as 0.033.

Another method of calculation, which should be more suitable for showing a correlation between characters due to environment, has also been tried. For each decade the means for Vert and Pd have been calculated, but instead of examining the correlation of these means I have first subtracted from them the probable values for Vert and Pd respectively which, according to the values known for their mother, could be expected in the offspring. In the resulting difference we have thus partly eliminated the inherited qualities. There is therefore a greater chance that these figures should show a negative correlation, and if so it would signify that, as the altered environments have, on the whole, influenced the number of Vert and Pd in opposite directions, we could also see in the individual fraternities that a great value for Vert was accompanied by a small one for Pd and conversely. But neither by this treatment of the deviations from the normal regression lines is any correlation found between corresponding deviations. From the 87 decades for which the values of mothers are known the average deviation from the regression lines is for Vert 1.218 and for Pd —0.314. The St. D. of the deviations from these means are 1.393 and 0.634 respectively and the correlation coefficient of the deviations is -0.006 ± 0.073.

It appears therefore as though the environmental conditions must be considered quite constant for all the fraternities or that the change in Vert and Pd is caused by different environmental factors.

V. Summary.

In an investigation of *Zoarces* from Station Langholm, which represents a population quite distinct from the Nakkehoved population, we have found that the laws of heredity for number of vertebrae, of rays in the right pectoral fin, and of pigment spots in the dorsal fin are statistically described by the same constants which we found for the material from Nakkehoved previously examined. The fraternal regressions curves considered are decidedly linear, and the maternal regression curves shew no significant deviations from the linear form.

It has thus been confirmed that the maternal, as well as the fraternal, correlation coefficient for the characters here examined in *Zoarces*, have sensibly smaller values than those which have been found on the whole for physical characters in the higher mammals.

An effort to answer the problem whether offspring from two annual groups of *Zoarces*, which differed widely in the average number of rays in the pectoral fins, shew a corresponding deviation has not afforded decisive results. The means for offspring of mothers with the same somatic values belonging to such two annual groups seem to suggest that the mothers belonging to the annual group which has the greater mean value have a smaller generative value than have the mothers of the other group. Nor did the means for the two groups of offspring show as great a difference as would have been expected when all the mothers had belonged to one annual group and the mothers of the two groups had been selected solely according to somatic values of mothers. On the other hand the offspring coming from mothers belonging to the annual group with the larger mean have still the larger mean value.

In the transplantation of a sample from Nakkehage the means, especially for vertebrae and rays of the pectoral fins, were greatly influenced, but otherwise it is only in the standard deviation and the fraternal correlation coefficient of the last named character that we have found values differing from those which statistically describe the laws of inheritance under natural conditions. For this character the standard deviation for offspring has, however, greatly increased and the distribution of frequencies is sensibly skew, further the fraternal correlation coefficient is much greater than usual, the increase in the deviations of means for various fraternities being proportionately greater than the increase of the deviation within the single fraternity. Although the means for number of vertebrae and of rays in the pectoral fin have been influenced in opposite directions by the transplantation, no individual negative correlation could be traced for the two characters, and neither was there any negative correlation found between means for fraternities when they were corrected according to heredity from mothers.

VI. Tables.

Table 17. Langholm. Vertebrae. Maternal Correlation 1915—1920 (631 mothers).

		Mothers												Total
		114	113	112	111	110	109	108	107	106	105	104	103	102	
Offspring	114	»	»	»	»	2	»	»	»	»	»	»	»	»	2
	113	»	»	»	1	5	2	2	»	»	»	»	»	»	10
	112	1	3	8	15	19	11	8	4	3	2	»	»	»	74
	111	1	2	11	25	36	40	38	16	8	7	1	»	»	185
	110	1	6	20	30	77	106	101	47	23	9	5	2	»	427
	109	2	»	18	35	72	129	149	86	52	17	8	5	»	573
	108	»	4	11	32	62	135	212	117	79	39	12	10	1	714
	107	»	»	6	11	35	105	118	115	66	46	14	3	1	520
	106	»	»	3	10	13	48	66	79	61	27	12	9	2	330
	105	»	»	3	2	4	21	24	34	42	27	12	8	2	179
	104	»	»	»	3	»	16	14	10	16	11	7	5	2	84
	103	»	»	»	1	»	1	7	4	3	8	4	8	»	36
	102	»	»	»	»	»	1	1	3	1	2	4	»	2	14
	101	»	»	»	»	»	»	»	»	»	»	1	»	»	1
	100	»	»	»	»	»	»	»	»	1	4	»	»	»	5
	99	»	»	»	»	»	»	»	»	»	»	»	»	»	»
	98	»	»	»	»	»	»	»	»	»	1	»	»	»	1
Total		5	15	80	165	325	615	740	515	355	200	80	50	10	3155

Table 18. Langholm. Pd. Maternal Correlation 1915—1920 (640 mothers).

		Mothers					Total
		21	20	19	18	17	
Offspring	21	4	22	11	1	»	38
	20	24	362	471	70	2	929
	19	11	447	1155	282	3	1898
	18	1	44	200	80	»	325
	17	»	»	8	2	»	10
Total		40	875	1845	435	5	3200

Table 19. Langholm. Pigment D_1. Maternal Correlation 1916—1919 (304 mothers).

Offspring	Mothers 18	17	16	15	14	13	12	11	10	9	Total
18	»	»	»	»	»	1	»	»	»	»	1
17	»	»	2	4	5	5	3	2	»	»	21
16	»	1	8	8	16	11	8	8	1	»	61
15	1	»	18	53	33	37	33	22	2	2	201
14	1	6	21	58	84	118	96	43	19	4	450
13	2	9	24	58	96	156	150	79	47	10	631
12	»	4	2	45	94	177	182	120	75	18	717
11	3	»	3	17	43	75	135	123	73	19	491
10	3	»	2	8	35	31	64	92	47	18	300
9	«	»	»	7	4	17	28	43	25	15	139
8	»	»	»	2	»	2	11	7	1	4	27
7	»	»	»	»	»	»	»	1	»	»	1
Total	10	20	80	260	410	630	710	540	290	90	3040

Table 20. Langholm. Vertebrae. Distribution of Frequencies for Embryos.

Sampl. tak. in	1915	1916	1917	1918	1919	1920
Vert.	Freq.	Freq.	Freq.	Freq.	Freq.	Freq.
114	4	»	»	»	2	»
113	7	2	1	1	4	4
112	59	13	15	20	18	12
111	127	29	31	49	43	42
110	313	60	86	129	79	98
109	396	110	131	146	116	131
108	403	119	164	172	132	169
107	310	111	114	116	99	137
106	181	65	88	61	64	80
105	78	40	54	27	33	43
104	46	18	31	10	13	18
103	12	7	9	8	7	8
102	3	5	3	1	4	5
101	1	1	»	»	»	»
100	»	»	3	»	1	2
99	»	»	»	»	»	»
98	»	»	»	»	»	1
Total	1940	580	730	740	615	750
$\sigma_2{}^2$	3.526807	3.914875	4.022061	3.209312	4.090650	3.901193

Table 21. Langholm. Pd. Distribution of Frequencies for Embryos.

Sampl. tak. in	1915	1916	1917	1918	1919	1920
Pd.	Freq.	Freq.	Freq.	Freq.	Freq.	Freq.
21	42	2	»	1	33	8
20	755	94	120	178	306	221
19	1097	348	482	495	272	486
18	76	141	126	66	4	43
17	»	5	7	»	»	1
Total	1970	590	735	740	615	750
$\sigma_2{}^2$	0.357098	0.437693	0.372049	0.311403	0.360648	0.341703

Table 22. Langholm. Pigm. D_1. Distribution of Frequencies for Embryos.

Sample taken in	1916	1917	1918	1919
Pigm. D_1.	Freq.	Freq.	Freq.	Freq.
18	3	1	»	»
17	17	13	5	»
16	31	38	21	5
15	87	97	80	52
14	171	210	187	148
13	252	334	257	255
12	253	304	304	301
11	180	228	180	223
10	118	141	86	128
9	59	59	34	82
8	8	5	5	24
7	1	»	1	2
Total	1180	1430	1160	1220
$\sigma_2{}^2$	3.314289	2.936304	2.503983	2.699290

Tabel 23. Langholm. Vertebrae. Distribution of Frequencies for Means of Siblings.

Sample taken in	1915	1916	1917	1918	1919	1920
Vert.	Freq.	Freq.	Freq.	Freq.	Freq.	Freq.
111.5	2	»	»	1	2	1
111.1	1	2	»	2	5	2
110.7	4.5	2	»	2	2	1
110.3	8	4	4	5	1	5
109.9	16.5	3	10	14	8	4
109.5	16	8	5	13	6	9
109.1	15.5	7	18	17	11	14
108.7	23.5	9	14	14	16	14
108.3	24.5	15	11	15	11	17
107.9	18.5	10	16	17	17	22
107.5	17.5	11	11	13	11	13
107.1	17	15	18	15	8	13
106.7	9	8	9	8	8	10
106.3	9	10	5	4	4	11
105.9	3.5	3	10	3	2	4
105.5	4	4	7	3	5	3
105.1	1.5	1	5	1	1	3
104.7	1.5	2	1	»	1	2
104.3	»	»	1	1	3	1
103.9	0.5	1	»	»	1	»
103.5	0.5	1	»	»	»	»
103.1	»	»	»	»	»	»
102.7	»	»	1	»	»	»
102.3	»	»	»	»	»	»
101.9	»	»	»	»	»	»
101.5	»	»	»	»	»	1
Total	194	116	146	148	123	150
σ_q^2 [1]	1.915828	2.205220	2.227540	1.722825	2.482194	2.158260

[1] Calculated from exact means.

Table 24. Langholm. Pd. Distribution of Frequencies for Means of Siblings.

Sampl. tak. in	1915	1916	1917	1918	1919	1920
Pd.	Freq.	Freq.	Freq.	Freq.	Freq.	Freq.
21.0	»	»	»	»	1	»
20.8	0.5	»	»	»	1	»
20.6	0.5	»	»	»	1	»
20.4	3	1	»	»	2	»
20.2	4.5	»	»	»	8	3
20.0	14.5	2	1	4	9	9
19.8	23.5	6	5	4	26	8
19.6	32.5	5	10	13	26	12
19.4	23.5	7	10	32	18	28
19.2	39.5	16	31	26	18	35
19.0	37	31	33	37	11	33
18.8	12.5	12	23	19	2	11
18.6	3.5	13	13	9	»	6
18.4	2	9	13	2	»	3
18.2	»	9	3	2	»	»
18.0	»	5	4	»	»	1
17.8	»	1	1	»	»	1
17.6	»	1	»	»	»	»
Total	197	118	147	148	123	150
σ_q^2	0.168215	0.257354	0.173953	0.122213	0.161623	0.166772

Table 25. Langholm. Pigm. D_1. Distribution of Frequencies for Means of Siblings.

Sampl. tak. in	1916	1917	1918	1919
Pigm. D_1	Freq.	Freq.	Freq.	Freq.
16.0	»	1	»	»
15.7	»	»	»	»
15.4	»	»	»	»
15.1	4	3	»	»
14.8	1	»	»	1
14.5	5	2	1	»
14.2	3	3	3	1
13.9	3	8	7	2
13.6	8	10	9	5
13.3	7	13	12	6
13.0	8	14	11	7
12.7	14	8	13	13
12.4	12	16	12	20
12.1	11	10	14	14
11.8	8	15	8	3
11.5	12	10	13	11
11.2	1	6	3	11
10.9	7	12	5	10
10.6	7	5	3	7
10.3	2	6	2	3
10.0	2	1	1	1
9.7	2	»	»	2
9.4	»	»	»	3
9.1	1	»	»	2
Total	118	143	117	122
σ_q^2 [1]	1.686153	1.391759	0.906310	1.258799

[1] Calculated from exact means.

Table 26. Langholm 1920-Sample. Vertebrae. Fraternal Correlation (150 Groups of siblings).

	113	112	111	110	109	108	107	106	105	104	103	102	101	100	99	98	Total
113	2	2	1	6	2	1	1	1	»	»	»	»	»	»	»	»	16
112	2	6	13	8	8	5	2	3	1	»	»	»	»	»	»	»	48
111	1	13	32	35	33	28	17	4	2	3	»	»	»	»	»	»	168
110	6	8	35	82	92	87	51	20	9	2	»	»	»	»	»	»	392
109	2	8	33	92	132	111	76	44	15	6	4	1	»	»	»	»	524
108	1	5	28	87	111	174	160	65	24	13	3	2	»	2	»	1	676
107	1	2	17	51	76	160	122	67	33	12	5	2	»	»	»	»	548
106	1	3	4	20	44	65	67	50	41	14	5	6	»	»	»	»	320
105	»	1	2	9	15	24	33	41	22	14	8	3	»	»	»	»	172
104	»	»	3	2	6	13	12	14	14	2	5	1	»	»	»	»	72
103	»	»	»	»	4	3	5	5	8	5	»	2	»	»	»	»	32
102	»	»	»	»	1	2	2	6	3	1	2	»	»	2	»	1	20
101	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»
100	»	»	»	»	»	2	»	»	»	»	»	2	»	2	»	2	8
99	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»
98	»	»	»	»	»	1	»	»	»	»	»	1	»	2	»	»	4
Total	16	48	168	392	524	676	548	320	172	72	32	20	»	8	»	4	3000

Table 27. Langholm 1920-Sample. Pd. Fraternal Correlation (150 Groups of siblings).

	21	20	19	18	17	16	Total
21	»	20	12	»	»	»	32
20	20	404	412	8	»	»	844
19	12	412	1404	112	2	2	1944
18	»	8	112	50	1	1	172
17	»	»	2	1	»	1	4
16	»	»	2	1	1	»	4
Total	32	844	1944	172	4	4	3000

Table 28. Langholm 1917-Sample. Pigment D_1. Fraternal Correlation (143 Groups of Siblings).

	18	17	16	15	14	13	12	11	10	9	8	Total
18	»	1	1	3	3	1	»	»	»	»	»	9
17	1	14	15	25	20	17	12	8	3	1	1	117
16	1	15	34	64	80	73	32	26	8	9	»	342
15	3	25	64	122	240	228	114	56	16	5	»	873
14	3	20	80	240	402	524	354	163	72	27	5	1890
13	1	17	73	228	524	812	672	437	167	68	7	3006
12	»	12	32	114	354	672	722	493	243	88	6	2736
11	»	8	26	56	163	437	493	432	322	108	7	2052
10	»	3	8	16	72	167	243	322	296	132	10	1269
9	»	1	9	5	27	68	88	108	132	86	7	531
8	»	1	»	»	5	7	6	7	10	7	2	45
Total	9	117	342	873	1890	3006	2736	2052	1269	531	45	12870

Table 29. Nakkehage Experiment of Transplantation 1917. Vertebrae. Maternal Correlation.

		Mothers										Total
		116	115	114	113	112	111	110	109	108	107	
Offspring	120	1	1	»	3	»	»	»	»	»	»	5
	119	2	1	1	9	1	3	»	»	»	»	17
	118	6	9	3	11	5	4	2	»	»	»	40
	117	17	17	23	6	13	14	2	»	1	»	93
	116	4	21	23	24	16	19	4	»	5	»	116
	115	9	30	38	24	33	26	3	»	4	1	168
	114	10	14	32	29	36	29	9	3	»	»	162
	113	»	19	24	13	26	28	10	4	»	3	127
	112	»	3	10	9	20	11	16	3	»	1	73
	111	»	4	4	1	8	6	15	5	»	1	44
	110	1	1	1	1	1	7	6	4	»	2	24
	109	»	»	»	»	1	3	3	1	»	1	9
	108	»	»	1	»	»	»	»	»	»	1	2
Total		50	120	160	130	160	150	70	20	10	10	880

Table 30. Nakkehage Experiment of Transplantation 1917. Pd. Maternal Correlation.

		Mothers				Total
		21	20	19	18	
Offspring	21	»	1	2	»	3
	20	7	41	88	1	137
	19	3	130	350	17	500
	18	»	28	138	42	208
	17	»	10	42	9	61
	16	»	»	6	1	7
	15	»	»	4	»	4
Total		10	210	630	70	920

Table 31. Nakkehage Experiment of Transplantation 1917. Pigment D_1. Maternal Correlation.

		Mothers								Total
		17	16	15	14	13	12	11	10	
Offspring	18	»	»	»	1	1	»	»	»	2
	17	»	1	7	3	4	»	»	»	15
	16	2	4	13	13	6	1	»	1	40
	15	5	11	19	17	22	6	1	2	83
	14	3	19	34	41	41	22	6	3	169
	13	5	21	30	43	55	25	6	1	186
	12	4	10	21	37	37	23	17	5	154
	11	»	4	15	15	18	20	13	3	88
	10	1	»	1	»	6	9	7	4	28
	9	»	»	»	»	»	3	»	1	4
	8	»	»	»	»	»	1	»	»	1
Total		20	70	140	170	190	110	50	20	770

Table 32. Nakkehage Experiment of Transplantation 1917. — Distribution of Frequencies for Embryos[1]).

Vert.	Freq.	Pigm.	Freq.
120	6	18	2
119	17	17	15
118	41	16	43
117	100	15	90
116	118	14	175
115	175	13	197
114	169	12	157
113	134	11	88
112	80	10	28
111	46	9	4
110	23	8	1
109	9		
108	2		
Total	920		800
σ_2^2	4.554130		2.556475

Table 33. Nakkehage Experiment of Transplantation 1917. — Distribution of Frequencies for Means of Siblings.

Vert.	Freq.	Pd.	Freq.	Pigm.	Freq.
118.0	1	20.0	3	16.1	1
117.5	1	19.7	4	15.8	»
117.0	3	19.4	12	15.5	1
116.5	7	19.1	19	15.2	2
116.0	8	18.8	23	14.9	1
115.5	10	18.5	13	14.6	1
115.0	9	18.2	4	14.3	2
114.5	14	17.9	4	14.0	7
114.0	8	17.6	7	13.7	16
113.5	13	17.3	»	13.4	13
113.0	6	17.0	2	13.1	7
112.5	3	16.7	1	12.8	5
112.0	3			12.5	5
111.5	2			12.2	5
111.0	3			11.9	4
110.5	1			11.6	4
				11.3	4
				11.0	1
				10.7	1
Total	92		92		80
$\sigma_q^{2\,2}$)	2.601847		0.436805		1.102475

[1]) For Pd. see Table 29.
[2]) Calculated from exact means.

Table 34. Nakkehage. Experiment of Transplantation 1917. Correlation between Vertebrae and Pd.

		Vertebrae													Total
		120	119	118	117	116	115	114	113	112	111	110	109	108	
Pd.	21	»	»	1	»	»	1	»	1	»	»	»	»	»	3
	20	1	5	8	18	16	30	20	14	14	5	4	1	1	137
	19	3	5	18	52	57	91	96	83	46	26	17	7	»	501
	18	1	5	7	22	33	40	38	32	18	8	2	1	»	207
	17	1	2	6	7	11	11	13	4	»	5	»	»	1	61
	16	»	»	»	»	1	1	2	»	1	2	»	»	»	7
	15	»	»	1	1	»	1	»	»	1	»	»	»	»	4
Total		6	17	41	100	118	175	169	134	80	46	23	9	2	920

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COMPTES-RENDUS

DES TRAVAUX

DU

LABORATOIRE CARLSBERG

14ME VOLUME.

No. 20

COPENHAGUE

EN COMMISSION CHEZ H. HAGERUP

IMPRIMERIE DE THIELE

1923

Prix: 1 Kr.

RAPPORT SUR LA DONATION HANSEN

(PAR L'ADMINISTRATION DE LA DONATION)

(Suite du rapport fait au Vol. II, p. 381)

PEU de temps après la première distribution du Prix Hansen, la grande guerre vint interrompre brusquement toute coopération entre les hommes de science des divers pays. Dans ces conditions, l'Administration de la Donation Hansen a jugé convenable, pour distribuer de nouveau ce prix, d'attendre qu'il se produisît dans l'état de choses une amélioration suffisante pour permettre de reprendre les communs efforts dans le domaine de la science.

Dans une séance tenue le 16 novembre 1921, l'Administration décida que la deuxième distribution du prix Hansen aurait lieu le 8 mai 1922, et que cette fois il conviendrait de le décerner à quelque savant parmi ceux qui s'étaient distingués par des travaux importants ayant trait à la microbiologie générale, non médicale. A la demande de l'Administration, M. le Dr *Calmette*, Directeur de l'Institut Pasteur, à Paris, et M. le Professeur et Dr *Theobald Smith*, à Princeton (États-Unis d'Amérique), voulurent bien, comme ils l'avaient fait pour la première distribution de ce prix, consentir à se joindre au Jury. Le troisième membre du premier jury, M. *Gaffky*, Geheimer Obermedizinalrat, à Berlin, était décédé dans l'intervalle qui séparait les deux distributions.

Le jury, présidé par M. le Prof. *C. O. Jensen*, prit la décision, à l'unanimité, de décerner le prix à M. le Dr *M. W. Beijerinck*, ancien Professeur de microbiologie à la Haute École technique de Delft (Hollande).

Comme M. le Professeur Beijerinck, pour cause de santé, n'était guère, à ce moment là, en état de se rendre à Copenhague pour y recevoir le prix, celui-ci lui fut remis, le 29 mai 1922, dans sa demeure, à Gorssel (Hollande), par le président de l'Administration.

Le secrétaire de la Donation, M. *J. L. Nathansen*, avocat de la cour supérieure à Copenhague, est décédé le 24 de l'année passée; sur notre demande, son associé, M. *V. Falbe-Hansen*, avocat de la même cour, a bien voulu se charger des fonctions de secrétaire.

En août 1922.

L'Administration de la Donation Hansen.

C. O. Jensen. **Johs. Schmidt.** **S. P. L. Sørensen.**

V. FALBE-HANSEN
Secrétaire de la Donation.

CROSSING-OVER BETWEEN THE X- AND THE Y-CHROMOSOME IN *LEBISTES*.

BY

O. WINGE.

In a paper on the conditions of inheritance in *Lebistes reticulatus* (1922 b), discussing sex-linked as well as one-sided male inheritance, I mentioned an observation of crossing-over between the X- and Y-chromosome in the male *Lebistes* and stated that further details would be published later on. It is the object of the present report to fulfill this promise.

In the work of Schmidt on *Lebistes* (1920), the first unquestionable case was brought forward on conditions of inheritance which pointed to factors connected with the Y-chromosome. The investigations made by me, at a later date, on the cytological conditions in *Lebistes* (1922 a), have shown that the diploid chromosome number was 46 in both sexes — and in my first-named paper I stated, as the most important result of my experimental cross-breedings that, notwithstanding the lack of any visible difference between the chromosomes themselves, the male *Lebistes* decidedly belonged to the XY-type and the female to the XX-type, such as is the case with *Drosophila melanogaster*. In effect, from the one-sided male inheritance, which could only be explained by assuming the existence of a factor-bearing Y-chromosome, one must logically conclude that X-chromosomes were also present and, thus, that the expression for the female chromosomes was $44 + X + X$, that of the male one $44 + X + Y$.

In full conformity with the above, I was able to prove sex-linked inheritance in at least one of the *Lebistes*-»races« I worked on, a further instance of the indubitable conformity of the results of genetical and cytological investigations.

At the time I communicated the first case of crossing-over between the X- and Y-chromosomes, — a phenomenon which had not hitherto been brought to light for the simple reason that no factor-bearing Y- (or W-) chromosome had hitherto been known with certainty to exist — I had no idea that another person was studying the same matter and had simultaneously arrived at the same results as I.

The Japanese scientist, Aida, has undertaken extensive experimental crossings with another species of fish, the *Aplocheilus (Haplochilus) latipes*, belonging to the Poeciliidae family, published in »Genetics«, vol. 6, 1921, p. 554[1]). In this paper, he not only proves the one-sided male inheritance, but also the crossing-over between the X- and the Y-chromosome, so that our two observations must be said to cover each other. However, no cytological investigation of the material worked on by Aida has been published as yet.

Like in my own case, Aida has studied colour-factors which he found to be present in the autosomes as well as in the sex-chromosomes. In one case, he has discovered homozygoty for a factor R (red), i. e. the »race« concerned carries the R-factor in the X- as well as in the Y-chromosome. In cross-over individuals segregated from heterozygotic males, the conditions of inheritance are exactly as would be expected with the supposition that the factor in question (R) has been transferred from the X- to the Y-chromosome.

While, in *Lebistes*, the colour-factors manifest themselves in male individuals only, the inheritance being phenotypically sex-limited to the male — a fact which has not made my researches any easier — the colour-factors appear in both sexes of *Aplocheilus*. There is another slight divergency in our investigations, viz: while the *Lebistes*-factors influence patterns and various colours in certain parts of male individuals, the *Aplocheilus*-factors involve a, so to speak, total differentiation of colour in the individuals.

Hitherto, only the R-factor (allelomorph r) has been found in the X- and Y-chromosomes af *Aplocheilus,* while in *Lebistes* the Y-chromosome has been proved to contain a whole series of

[1]) Said part of »Genetics« has only just appeared (Nov. 1922) and ought not to bear the date of Nov. 1921, as is the case. This misleading date of publication might create the impression that I ought to have been acquainted with Aida's work when, in May 1922, I sent my own paper in the press.

factors and the X-chromosome, until now, only one factor, s, which has been seen only in one instance to cross over, partially, to the Y-chromosome, said factor s being a complex factor composed of a series of linked factors. On the other hand, no colour-factors have, as yet, been found in the autosomes of *Lebistes*, whereas Aida has recorded the factor B and its allelemorphs B_1 and b.

In neglecting these minor differences, the results concerning the two species of fish must be said to cover each other exactly.

As to the genetic formulae, Aida and myself use unlike expressions to which I shall revert below, in connection with the presence-absence theory.

I shall now proceed to the relation of my experiments of the crossing-over between X- and Y-chromosomes.

Experiments on X-Y crossing-over.

In June 1917, while I was attached to the Carlsberg Laboratory, we received a male *Lebistes* which, in the main, had the appearance of the afore-mentioned (1922 b) »Spot Race« of which the males have the formula X_oY_m. The fish was bought from a Mr. Christensen, Poppelgade (= Poplarstreet), Copenhagen, who, in his turn, had received it from Berlin, in the summer 1916, from a dealer in aquaria.

This new type had the following characteristics in common with »Spot Race« males, viz: 1) large black spot in dorsal fin; 2) large red side-patch below and in front of dorsal fin; black dot near anal aperture; all being due to the *maculatus*-factor m in the male Y-chromosome. But whereas all the »Spot Race« males I had hitherto seen, possessed a rounded, colourless caudal fin, the above male had 4) a caudal fin with elongated upper edge and 5) vivid reddish-yellow colouring on upper and lower edge of caudal fin.

This »Poplarstreet« male N° 123, as it was named after the place where it was bought, was mated, on June 9, 1917, with a virgin female N° 125 without factors in the X-chromosomes and, therefore, corresponding to formula X_oX_o, whereupon the female bore, until Dec. 27, 1917, 6 broods numbering more than 200 young ones in all. 73 young males became adult, but only one of these had the elongated caudal fin of the »Poplarstreet«

male, all the others having the rounded colourless caudal fin of the »Spot Race«.

Accordingly, the factor e *(elongatus)*, responsible for the elongated caudal fin, must be supposed to reside in the male X-chromosome, which we know to pass on to the daughters; and we may also surmise that the single male with an elongated caudal fin owes its existence to the crossing-over, in a single case, of the e-factor to the Y-chromosome of a spermatocyte. The original »Poplarstreet« male having thus the formula X_eY_m, and therefore usually forming sexual cells carrying either X_e or Y_m, it must have happened at the crossing-over that, in an isolated case, the e-factor was transferred to the Y-chromosome and then linked to the m-factor there. The formula of the diverging male should therefore be X_oY_{em}.

The inference, that the crossing-over between the X- and the Y-chromosome has really taken place here, is fully confirmed by the following crossing-experiments undertaken as a check. The process of analysis is represented in fig. 1, under the form of a genealogical diagram.

As far as the non-cross-over individuals are concerned, the F_1-females of this category are supposed to have the formula X_oX_e and, in mating these females with F_1-males, the formula of which is X_oY_m, the outcome should be an equal number of males with elongated caudal fin, and without. In accordance with this, F_2 gave 26 X_eY_m and 27 X_oY_m. — The F_2-females were also expected to consist of two categories, partly X_oX_o, partly X_eX_o, and in fact, one F_2-female, mated with a »Spot Race« male (X_oY_m), produced only X_oY_m-males (11 in all) and therefore had the formula X_oX_o, while another F_2-female, mated with the same male, gave 4 males with elongated caudal fin (X_eY_m) and 3 without (X_oY_m); consequently, this latter F_2-female must have had the formula X_eX_o.

As said above, the non-cross-over males should, theoretically, have the formula X_oY_m, and their habitus entirely corresponded hereto, i. e. rounded caudal fin and the general appearance of the »Spot Race«. As a matter of fact, some such F_1-males, crossed with X_oX_o-females, produced exclusively round-tailed progeny. 22 sons in all were counted and none of these showed any trace of the e-factor.

It has thus been proved that the elongated caudal fin of the »Poplarstreet« race is due to a factor in the male X-chromosome and that, therefore, the original male must have had the formula

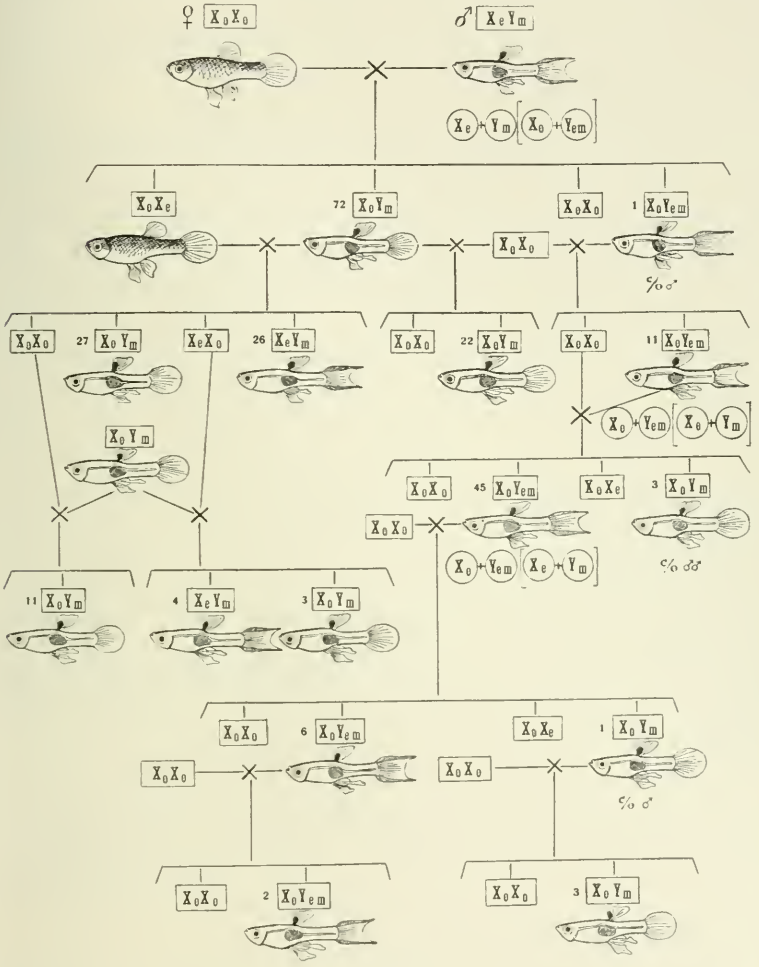

Fig. 1.

Genealogical survey showing crossing-over of factor e from the X- to the Y-chromosome.

X_eY_m. While the inheritance of factor m is one-sided male, the e-factor is of sex-linked inheritance.

We shall now examine the conditions of the diverging F_1-male with the elongated caudal fin, so as to find out whether its genetical behaviour corresponds to the hypothesis that the e-factor

has crossed over to the Y-chromosome, making the formula of the individual = X_oY_{em}.

The male progeny of such a male should, of course, always be provided with an elongated tail-fin, all the sons receiving the Y-chromosome. Only in case of a renewed crossing-over could the e-factor again be separated from the m-factor and round-tailed sons be segregated.

All the 11 sons of the diverging male proved to have elongated caudal fin, and this is already a proof that the e-factor has no longer its seat in the X-chromosome, but in Y and that, therefore, a crossing-over between X and Y must have taken place.

The sequel of the analysis gave the following result: The 11 X_oY_m-males were mated with some sisters which should correspond to formula X_oX_o, thus giving rise to a new generation, F_2, of cross-over males. As to the males, this generation consisted of 45 X_oY_{em} and 3 X_oY_m. In this connection, the 45 young ones are non-cross-over individuals, whereas the 3 are the result of a new crossing-over in which the Y-chromosome has again lost its e-factor, the latter having crossed back to the X-chromosome, its original seat.

Among the offspring of the 45 X_oY_{em}-males, as shown in fig. 1, was again found 1 round-tailed X_oY_m-male, the result of a new crossing-over. As might be expected, this male, when mated with an X_oX_o-female, reproduced exclusively its own type, while the other individuals of this and the following generation all have the elongated caudal fin and the formula X_oY_{em}.

Cross-over females are of course not so easily discerned as are cross-over males, as the females phenotypically show no colours at all. The only way in which we can recognize the factor-contents of a female individual is through crossing it with a male of well known genetic constitution.

In a single case I have found such a primary cross-over female. All the female progeny from a cross-over male, X_oY_{em}, with a female, X_oX_o, will be X_oX_o if no crossing-over between X and Y in the male happens. An X_oX_e female only can arise through crossing-over. — Among the F_3 offspring of the primary cross-over male, X_oY_{em}, with an X_oX_o female, 6 female individuals were analyzed by pairing them with X_oY_m-males (Spot Race). The five bore only X_oY_m males, 32 in all, and were therefore ordinary X_oX_o-females, but nº 6 has until now born 2 X_eY_m males and 3 X_oY_m and must

therefore have the formula X_oX_e, i. e. be a female individual arisen through crossing-over in an X_oY_{em} male.

We have thus proved that the e-factor, accountable for the elongated, coloured caudal fin of the original male individual, had its seat in the X-chromosome and was, therefore, of sex-linked inheritance (X_eY_m). By crossing-over between the X- and the Y-chromosome, it was, in one case out of 73, transferred to the Y-chromosome (X_oY_{em}), whereupon the inheritance of the elongated caudal fin became subsequently one-sided male. In the 4 male individuals out of 68, the Y-chromosome had again lost its e-factor which had anew crossed back to the X-chromosome, where it was found in a female individual.

An individual belonging, in all evidence, to the above mentioned „Poplarstreet Race", was bought in Sept. 1917, from another Copenhagen dealer in aquaria and was said to have arrived from Dresden one month earlier. The race was, in one and all, similar to the „Poplarstreet Race" and, after a cross with a „Spot Race" female X_oX_o, an offspring resulted, of which 22 were males, all with a rounded, colourless tail, showing that, in this individual also, the e-factor had its seat in the X-chromosome and that this male, therefore, also had the formula X_eY_m.

The whole matter will now appear as clear and we shall therefore discuss the deductions which might be made therefrom.

Theoretical considerations.

In the present and in a former report, I have shown that crossing-over between the X- and the Y-chromosome takes place in *Lebistes* and that the sole real difference between their X- and Y-chromosomes is that the Y-chromosome contains a dominant male factor, while the X-chromosome contains a recessive female factor. The e-factor, which produces the elongated, coloured caudal fin, may cross over from X to Y and vice versa, which, in other words, means that the male and the female factor are

evidently allelomorphs which, like other factors, may be exchanged between the chromosomes. Nor has a cytological examination revealed any morphological difference between the X- and the Y-chromosomes.

Therefore, if it be true that the Y-chromosome contains a special male factor, this factor must be rather strongly linked to the e-factor, for only one cross-over individual has been found among the 73 sons of an X_eY_m-male and 4 cross-over individuals among 68 sons of X_oY_{em}-males.

It is remarkable that crossing-over has not been equally frequent in both directions; however, as the amount of material was not very great, I do not feel justified in attaching any significance to this fact. Much stronger is, however, the linkage between the male factor and other factors found in the Y-chromosome, such as m, *(maculatus)*. i, *(iridescens)* etc. and the circumstance itself of diversity in the degree of linkage between sex- and colour-factors must be taken as evidence of a real gene, corresponding to the sex-factor, embodied in the chromosome and comparable with other genes. As to the m-factor, thousands of individuals, produced after mating of X_oX_o-females with X_oY_m-males, have been examined without one single individual with missing m-factor being found. This is very striking. At the outset, one sees no reason why said factors should be located so close to the male factor that no crossing-over can take place, but, as said before, it is quite possible that the male factor itself is identical with the m-factor, although in that case one must arrive at the conclusion that, with regard to sex-factors, multiple allelomorphs exist. Another explanation of the strong linkage af these colour-factors to the sex-factor would be that crossing-over is rather rare in *Lebistes*. As said in my earlier paper (1922 a), I have looked in vain for a typical stage of synapsis in the spermatocytes, and neither has diakinesis been observed. — Perhaps the explanation of the strong linkage might also be that only factors situated at some distance from each other can be exchanged, on account of a pronounced rigidity of the chromosomes.

It is certain that great difficulties oppose a close study of the topography of the Y-chromosome, for on one hand, the linkage between the coulour-factor and the sex-factor appears to be strong, while, on the other hand, the factors-contents of the female individuals cannot be directly ascertained. The future

must decide whether factors shall be found which are linked to the sex-factors in a sufficiently variable degree to make charting of the sex-chromosomes feasable. It is obvious that, in any case as far as *Lebistes reticulatus* is concerned, many colour-factors exist which have not yet been analyzed, for, although the variations of aspect within one single race are limited, a considerable number of *Lebistes*-types are found with dealers in aquaria as well as in the free nature.

I have mentioned that, in several of the *Lebistes*-races examinated, the X-chromosome is empty, e. g. in the "Spot Race", and has therefore been given the sign X_0. In this case, as well as in all the other *Lebistes*-races observed, the Y-chromosome alone contains a colour-factor. When I have compared this circumstance to the fact that the Y-chromosome of *Drosophila* is empty, the comparison is not entirely to the point, in as far as I have counted with the presence of a female factor in the X-chromosome of *Lebistes*, whereas the existence of a corresponding male factor in the Y-chromosome of *Drosophila* has not been evidenced. On the contrary, according to the latest, very interesting researches of Bridges, (1922), on flies with an anormal set of chromosomes, it seems as though the ratio between the numbers of autosomes and of X-chromosomes alone determines the sex in *Drosophila*.

At any rate, factors in *Lebistes* have been found, as yet, only in the sex-chromosomes, while not one has been discovered in the 22 autosomes. Notwithstanding this, the autosomes are undoubtedly factor-bearing, even if no special colour-factor exist in them. We do not know yet in how far species-specific chromatin is present in the chromosomes and whether it is common to all the chromosomes of one species. As only fundamental differences can be made the object of inheritance researches, nothing hinders the supposition that a great many species-specific factors exist homozygotically in all the biotypes of the species, and it is even probable that several chromosome-pairs contain, in principle, the same species-specific factors. This latter hypothesis seems to be confirmed to some extent by the fact that numerical progressions in the number of chromosomes are found within systematical genera of the vegetable reign, e. g. 7, 14, 21 *(Triticum)* or 9, 18, 27, 36, 45 *(Chrysanthemum)* etc., which progressions can only be explained by supposing that the higher

numbers are the result of the addition of two lower chromosome numbers after hybridization — or the doubling of the original chromosome-set. New polyploid species, originated by hybridizing two earlier species, with a simultaneous doubling of the chromosomes, — the probability of which I have established in previous researches (1917) — will have the genus-specific factors redoubled, but this feature is not traceable in the descendency of the new types, because they are homozygotic in the genus-specific factors, or even several times homozygotic. The fact that polymeric factors frequently occur in polyploid species — well known for instance from Nilsson-Ehle's studies of *Avena sativa* and *Triticum vulgare* — fully agrees with the supposition that the factors are repeated several times in the polyploid species; and it is quite natural to assume that even species which, on account of their number of chromosomes, do not reveal themselves as polyploid, contain many genus-specific factors which are common to all the chromosomes. Some chromosome researches seem to show that adhesion between originally freely "mendelating" chromosomes may take place; and a chronic conjugation of chromosomes, in connection with above conditions, will result in a gradual, manyfold appearance of the same factors in each single chromosome — or, in any case, in more than one.

According to this, all genera of a given family should be characteristic by the factors peculiar to that family, but they should be distinguished from each other by "genus-factors". All the species of a genus should contain identical factors, characteristic of that genus only, and furthermore a great many factors distinctive of the family, and be distinguished from each other only by a smaller number of species-specific factors. All the varieties within one species should, homozygotically, contain identical "species-factors" beside the particular genus- and family-factors which will probably be present in several or in all the chromosomes — and they should be mutually distinct by one or a few "variety-factors" having their seat in one or in a few chromosomes and disclosing themselves in Mendelian segregation, when the varieties are crossed.

As two varieties, chosen within a species, only differ as far as a few factors, the "variety-factors", are concerned, but are homozygotic and isogeneous with regard to all the species-, genus-, family- etc. factors, the effect of variety-factors is alone

observed in segregations. The greater the systematic unit under consideration, the more often must the genes, characteristic of that unit, be repeated in all the chromosomes.

Of course, one could not think of any absolute boundary between, for inst., "species-factors" and "variety-factors", but we must suppose that, in substance, those factors which are widely represented in all the chromosomes afford a basis for classification into large, systematic units; their frequent presence in the chromosomes will entirely prevent a segregation into types in which said factors are lacking and, with them, the corresponding morphological and physiological particularities; and the loss or the modification of a few such factors could hardly provoke any immediately visible mutation, because many others of the same nature would be left. At the most, this eventuality could be imagined to tend, throughout a long period of time, towards a slow shift in the characters of the species — a condition of things which might be associated with biological evolution.

The presence-absence theory. The denomination of factors.

According to the opinion of many geneticists on the nature of factors, the type holding the dominant factor contains a gene which is missing in the type holding the recessive factor. This theory has been named the "presence-absence theory«. This theory can be said to have given satisfaction until multiple allelomorphs were recorded, but after that it fell short, as the existence of a certain number of different types, all recessive to one and the same dominant type, could of course not be explained by the absence of the gene corresponding to the dominant factor. The notion imposed itself that, like the dominant type, the recessive type possesses a gene, but that this gene differs in value from that of the dominant type — and has a different value in the different recessive allelomorphs. According to this, allelomorphs should originate from genes, diverse in value, but having the same location in the chromosomes. This conception is unquestionably gaining ground.

It has been seen from my *Lebistes*-formulae that I use the denomination X_0 for an X-chromosome which contains no colour-factors, i. e. a mode of denomination in conformity with the original conception, — and this has been done deliberately.

Aida applies the formula R_xr_y to a male which I would designate by X_rY_o. In the first place, Aida uses X and Y as indexes showing the location of the factors considered and thereby gives more prominence to said factors. In my own work, I have made a rule of using the factors as indexes to the chromosome-denominations, for the reason that, to keep abreast with the recording of more and more factors in each chromosome, an expression as, for instance, $AAX_{rspn}Y_{rv}$ admits, according to my idea, of more rapid discrimination than $AAR_xR_yS_xP_xN_xV_y$. With my system, the factors and their location in each of the chromosomes are seen at a glance. As the charting of autosomes progresses, their factors could be represented in the same manner, e. g. $I_{thl}II_{bcd}$, etc. and the factor-set of a diploid type by $I_{tthhl}II_{bbccdd}$, this latter example corresponding to a homozygotic individual. In consequence, I would first of all change Aida's denomination, R_xr_y, into X_RY_r.

Furthermore, Aida has chosen R to represent the factor for the red colour, as most geneticists would have done, and r for the recessive allelomorph of this factor, whereas it would have been more descriptive to denote the factor for red by r (or R) and its absence by o; whether R or r should be chosen, would depend on whether red is dominant or recessive in relation to the normal type. My reason for choosing o to denote the absence of the colour-factors is that precisely the study of *Lebistes* (and *Aplocheilus*) very decidedly tend to bring about the conception that, whichever be the colour-factor considered, its allelomorph will be equal to o, i. e. that the corresponding gene is missing in the "recessive" type or is, in any case, inactive.

I have shown that, as crossing-over takes place between them, the X- and the Y-chromosomes are partners to the same extent as the other chromosomes in *Lebistes*. According to the current notion, each factor in the Y-chromosome should therefore have a corresponding allelomorph in the X-chromosome, and vice versa. Thus, the factors which I have called m, i, f, r, s and e should all have their corresponding allelomorph factors in the chromosome partner; but we have seen in the crossing experiments that whether we take a female in which m is lacking, or one in which i is laking — i. e. whether the female belongs to the "Spot Race" or to the "Old Race" — it contains the same factors, that is to say none (X_oX_o) from the accountant's point of view, as far as

colour-factors are concerned, or, in other words, only inactive or neutral genes. According to the current mode of denomination, my *Lebistes* females should, in one case, have the formula X_mX_m, i. e. those of the "Spot Race", where the males should be called X_mY_M; but if belonging to the "Old Race", their formula would be X_iX_i and that of the males X_iY_I; but whether we use the females of one race or the other, the result is exactly the same; the two kinds of females are identic as to inheritance, i. e. they are $X_{mi}X_{mi}$, a formula which is in so far irrational as m = i and equal to all the other "recessive" allelomorphs, which are all equal to o.

This discussion touches the question of the right conception and denomination of genes or factors; much would undoubtedly be gained if a uniform mode could be agreed upon.

In *Drosophila*. the organism which has hitherto been the most extensively studied, the "normal type", i. e. the wild type, is the established base upon which the whole terminology of factors has been built up. When forms appear, which are recessive with regard to this base, they are denoted by the corresponding small letters, while new dominant forms are denoted by capitals.

In *Antirrhinum*, there is no such decidedly normal type to refer to, and we might say that, here, the denomination of factors generally refers to an entirely recessive type.

In reality, the results are alike. In both organisms, the value of the factors is established in relation to a given type of fixed habitus or — in case the entirely recessive type is not viable — in relation to a type, the aggregate characters of which have been determined theoretically.

It makes no difference whether the type in question is measured or characterized in comparison with a wild type, chosen as "normal", or with an entirely recessive type. In both cases, the notation is relative, because it implies reference to a type, an individual of the same species, with which all other types are then compared.

Genetical science does not seem to take any interest in the systematic basis of the species as such. But we should say that this is an appearance only.

The present system of denoting factors — or rather pairs of factors — unavoidably presupposes that all types belonging to a species

possess the same number of genes: any type must either possess the recessive or the dominant allelomorph of any pair of factors.

On reconsideration, this is a rather curious idea, which immediately calls forth the question as to whether all the species of a genus, all the genera of a family, etc. have the same number of genes. Logically, and from a formal point of view, this hypothesis need not yet run against any self-contradiction. Taking *Antirrhinum* as an instance, and F to stand for the red of its flowers, there could be no objection to the use of ff, not only for all ivory-coloured *Antirrhinum*, but also for all white peas or all animal organisms; for, in fact, none of the latter possess the predisposition to red of the *Antirrhinum* corolla. This, precisely, shows that, in the study of inheritance, the tendency to formalism is becoming rather pronounced.

There is hardly any reason whatever to suppose that two different varieties possess an equal number of inheritance-elements, i. e. genes. Even as the different species of a genus may hold chromosomes of entirely differing number and aspect, and therefore — if our conception of the genes, as arranged along the longitudinal axis of the chromosomes, is to hold at all — must have a widely differing number of genes, so it must no doubt also apply to the varieties within the species, that they need not contain the same number of genes.

The mistake we make in continually considering the number of genes as constant and identical in all related (and unrelated) organisms, very likely derives from the fact that our conception of the allelomorphs is somewhat erroneous and that, in principle, we must revert to the old presence-absence theory which, alone, explains why a type, a variety, a species, etc. can hold certain genes that are entirely lacking in others, where not even a corresponding gene-partner is found.

Not only in this connection do we get the impression that the presence-absence theory holds good. This applies to almost any genetical question. Of course, it makes no great difference whether, to explain a recessive factor, we assume the existence of a gene which is, under all circumstances, inactive, or whether we simply deny the existence of any such gene. But I suppose that the distinction between non-differentiating and differentiating elements really ought to find its expression by attributing the denomination „genes" exclusively to the latter, while the former

should, from a genetical point of view, be represented by zero (0), as having no traceable influence on the sum of inherited elements of the organism. In reality, it is perhaps unessential whether the factor-denomination is assigned to the dominant or to the recessive allelomorph (its partner being put equal to 0). At any rate, it will not always be possible to decide which one of the two allelomorphs should have the factor index. It seems natural, however, as all recessive allelomorphs, as m, i, f, r, etc., have the same indeterminate value, to give them the value zero; in fact there is no difference between them, neither relatively nor absolutely. Although the study of inheritance essentially concerns relativity and fundamental differences, the real and, to my opinion, indispensable basis, would be generally to take 0 as the value of the recessive factors or genes and to count with positive values of recessive factors or genes only in such more rare cases as, for example, multiple allelomorphs. Referring to the latter case, one could imagine some slight shift in the chemical nature of the gene — provoking a special activity of each of the allelomorphic genes — to be the cause of the appearance of multiple allelomorphs; and in the first case, a shift in the chemical nature of the gene, to such an extent that the gene entirely lost its differentiating influence on the type.

In spite of this opinion, I have no doubt but that it would generally be inconvenient to apply another mode of denomination to the factors, than the present one. Zero as an index for the lack of a factor, would often be less suitable than a small letter, partly, because one is not always able to decide whether an allelomorphic factor is really missing in the case under consideration, partly, because the use of a small letter would often give a clearer formula. — On the other hand, it would be convenient, when comparing to a "normal type", to be able to symbolize the factor, characterizing a different and recessive type, by a small letter, for inst. a, the allelomorph of the normal type being here equal to 0, and to symbolize the factor of a dominant type by a capital letter, say, B, the allelomorph of the normal type being again 0.

Although, for my work on *Lebistes*, I have tentatively used a mode of denomination which was, in my opinion, more correct than the one used hitherto, I am quite aware that this method might cause some inconvenience in certain cases. The future must decide upon the system to be preferred.

As said before, there can hardly be any question of dominance or recessivity as far as the colour-factors in *Lebistes* are concerned. In the female, no colour-factor whatever can be traced; and in the male, when comparing to *Drosophila* conditions, even recessive factors would evidently appear and, *a fortiori*, the dominant ones. As however, at variance with *Drosophila*, crossing-over between X- and the Y-chromosome is possible in *Lebistes*. we might say that the colour-factors dominate their recessive allelomorphs but, as the latter are equal to 0, the index for dominance does not mean very much in this connection, as in many others; — it would simply mean that the gene, if present at all, manifests itself phenotypically in the male.

Considering the sex-factors in *Lebistes*, it is possible that real allelomorph factors exist, i. e. the male and the female factors, although we might conceive that, here again, the male sex-factor alone acts positively, whereas the female factor is 0, i. e. does not alter the female character in male direction.

It will be one of the tasks of the future to determine the dimensions of the genes. By calculating the percentage of crossing-over in *Drosophila*. the location of a great many of its chromosome-factors has already been charted, taking it for granted that the cross-over percentage may still be considered mainly as some function of the distance separating said genes along the longitudinal axis of the chromosome. The dimensions of the chromosomes being known, as well as the number of factors recorded and the relative distance between these, one may vaguely conceive the smallness of the units of measurement which will have to be used in connection with the genes. Probably, the length of chromosome to be considered in such calculations should be that characteristic of the synapsis stage, or an adjoining stage; it is probable that the genes of the chromosomes we observe, for example during the heterotypical division, are not strung along a straight line, but bunched like the pearls of a row kept in a wide test tube or the like. Unfortunately, the length of the chromosome at the stage of synapsis cannot be easily measured; a rough estimate will, however, show that we are still far from dimensions as for inst. those of the molecules of albumen.

Even from a slight crossing-over as, say, 0.2, between two strongly linked genes, we can conclude nothing as to the size of the genes, for two genes may of course be in close vicinity,

whether they are large or small. It is necessary to know at least three neighbouring genes before being able to estimate the dimensions of the space occupied by the middle gene, — and it would be important that more observations be made of such strongly linked genes, for the furtherance of the knowledge of the size of the genes. By the way, we do not know whether the genes take up the entire length of the longitudinal axis of the chromosome.

Sex-linked, sex-limited and one-sided inheritance.

As a contribution towards the adoption, in scientific literature, of uniform terminology for the various modes of inheritance (at present, such terms as "sex-limited" and "sex-linked" are often used a random), I would point out that the denomination "sex-linked" (geschlechtsgebunden) ought to be applied only to conditions of inheritance explainable by the presence of the factor in question in those sex-chromosomes which are normally found in individuals of both sexes (X- and Z-chromosomes). "One-sided" (einseitig) inheritance should be used only in such cases where the factor in question has its seat in that sex-chromosome which is normally found in one sex alone (Y- and W-chromosomes), adding the mention male or female so as to specify whether said factor belongs to the Y- or to the W-chromosome. The value of this distinction is not lessened by the fact that crossing-over between X- and Y-chromosomes has been proved, for nothing opposes the notion that, through a genetic displacement, one mode of inheritance may change into another. As to the denomination "sex-limited" inheritance, we are less fortunate because the secondary sex-characters, to which the term is generally assigned, are usually due to autosome-factors which, however, produce exclusively phenotypical effect in individuals of one of the sexes. The milking capacity in cattle, the production of lupuline in hop, are instances of so-called (female) sex-limited inheritance. In reality, it is not a question of any special mode of inheritance, so far as we know, but only of phenotypical differences dependent on sex-determination. — For this reason, the expression "sex-limited inheritance" is not very judicious in any case. "Sex-limited manifestation" would be better. "Phenotypically sex-limited inheritance" might perhaps do.

Résumé.

A factor e *(elongatus)*. involving an elongated caudal fin in male individuals of *Lebistes reticulatus*. shows sex-linked inheritance, for which reason the e-factor must have its seat in the X-chromosome of the male.

Crossing-over between the X- and the Y-chromosome is recorded, the e-factor thereby being transferred from the X-chromosome to the Y-chromosome. Males having thus their e-factor in the Y-chromosome, show one-sided male inheritance of the elongated tail fin.

In these cross-over males, having the e-factor in the Y-chromosome, a new crossing-over may take place between X and Y, so that the e-factor returns to the X-chromosome, and so on. The mode of inheritance is therefore continually oscillating between sex-linked inheritance and one-sided male inheritance.

The above shows that the X- and the Y-chromosome in *Lebistes* respectively contain a dominant male sex-factor and a recessive female sex-factor; also that this pair of factors is entirely like other pairs, because its location in the chromosome is certain, and because it shows linkage to colour-factors, crossing-over, etc. In above respects, the divergence from conditions in *Drosophila melanogaster* is rather striking.

The presence-absence theory and the ideal factor denomination are discussed.

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